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METABOLISM OF *SCHIZOTRYPANUM CRUZI* CHAGAS. I. EFFECT OF CULTURE AGE AND SUBSTRATE CONCENTRATION ON RESPIRATORY RATE.*

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Since the first description of *Schizotrypanum cruzi* by Chagas in 1909, it has been apparent that the organism occupies a unique position in the Trypanosomidae. Physiologically, *S. cruzi* differs in many respects from species thought to be closely related, e.g. *Trypanosoma lewisi,* and those more distantly related, e.g. *T. gambiense* and *T. rhodesiense.* The most obvious difference between *S. cruzi* and other species of trypanosomes is the inability of the blood stream forms of *S. cruzi* to undergo multiplication. Whether trypanosome stages undergo division in the invertebrate host and in cultures is not agreed upon (Diaz, 1939; Elkeles, 1951). Another difference between *S. cruzi* and many other trypanosomes is the ease with which it can be grown in vitro in a wide variety of media (Weinman, 1953; Silva, 1955).

Earlier literature on the energy metabolism of *S. cruzi* was reviewed by von Brand (1952). Important additional studies have been carried out by Baernstein (1953a, 1953b), Agosin and von Brand (1955), von Brand and Agosin (1955), Agosin and Weinbach (1956), Ryley (1956), Fulton and Spooner (1959), and Zeledon (1959).

Because of certain quantitative discrepancies in the work of several authors, the present study is concerned with respiratory metabolism of *S. cruzi* under defined conditions of cultivation, with particular reference to age. The changes in endogenous respiration with respect to age of culture, the ability of glucose to stimulate respiration of organisms in different phases of the growth curve, and the ability of *S. cruzi* culture forms to utilize glucose at low concentration, have been examined.

MATERIALS AND METHODS

Parasites used in this study were derived from the Brazil strain of *Schizotrypanum cruzi,* obtained from Dr. John F. Kent, Walter Reed Army Medical Center. The flagellates were grown on two media. The first, the diphasic medium of Senekjie (1943) as modified by Tobie, von Brand, and Mehlman (1950), was used for routine transfer of stock cultures. Flask cultures used for metabolic studies were prepared in the following manner: 55 ml of blood agar base in a 500 ml Erlenmeyer flask was overlain with 50 ml of sterile Locke's solution. The flask was then inoculated with 1 ml of medium containing about 30 to 50×10^6 organisms from a 7-day-old

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stock tube culture. Cultures were maintained at 26 C. Also used in the present study was a chemically undefined liquid medium, hereafter referred to as HM medium. This was prepared as follows: Brain-heart infusion (Difco) was brought to a boil and defibrinated sheep's blood added in a final concentration of 10 percent. The blood was allowed to coagulate and the mixture shaken while hot. The first 100 ml of filtrate was refiltered and was free of particles. The filtered medium was dispensed in appropriate cotton-stoppered vessels and autoclaved for 15 minutes at 120 C. This medium can be stored in closed containers at room or refrigerator temperature for periods of at least 2 months. Stock cultures were carried in test tubes 18 mm × 150 mm containing 10 ml of medium. Cultures used for metabolic studies were grown in 50-ml centrifuge bottles with tapered necks. Two ml of stock culture material (10 to 12 days of age) was inoculated into 35 ml of medium. Cultures were also grown in 100- to 250-ml quantities in erlenmeyer or florence flasks for some experiments.

For manometric studies, organisms were harvested from diphasic flask cultures by pooling the fluid portion of three or more cultures and filtering through four thicknesses of cotton gauze. The parasite-medium suspension was then centrifuged at 80 *g* for 1 minute. The agar-free parasite suspension was removed and the pellet was discarded. The suspension was centrifuged at 600 *g* for 15 minutes and the supernatant discarded. The pellet was washed three times by centrifugation with 20 ml of Krebs-ringer phosphate (KRP) solution (pH 7.2) and centrifuged for 15 minutes at 600 *g*. The thrice-washed parasites were resuspended in KRP for distribution to experimental vessels. The above was carried out at room temperature. Organisms were harvested from the all-liquid medium by pooling the contents of at least six centrifuge bottles and centrifuging at 1000 *g* for 5 minutes. The supernatant was discarded and the pellet resuspended in about 30 ml KRP solution (pH 7.2). The parasites were washed 3 times and sedimented again at 1000 × g for 5 minutes. The pellet was resuspended in KRP to yield the final parasite concentration desired for manometric measurements. All preparative operations with cells from HM was carried out at 2 C.

Oxygen consumption was determined with conventional Warburg respirometers using double-sidearm flasks of approximately 20 ml volume with 20 percent KOH in the center well. The main compartment contained 2.6 ml of parasite suspension. Measurements were made at a temperature of 32 C ± 0.02 C. The vessels were agitated at approximately 110 oscillations per minute through an amplitude of ca 4 cm. Temperature equilibration was carried out for 10 minutes. The frequency of readings depended upon the total amount of respiration occurring during the reading interval. Endogenous respiration was recorded until at least 10 microliters of O_2 had been consumed. The substrate was added to the main compartment from the side arm in a volume of 0.2 ml. In control vessels blank additions to the main compartment were made from the side arm. One ml of the parasite suspension was diluted to 50 or 100 ml in a volumetric flask for determining the parasite concentration. Parasite concentration was estimated as the average of six haemocytometer counts. In some experiments aliquots of parasite suspensions were stored at −20 C for nitrogen determinations which were carried out on duplicate samples, using a modified Kjeldahl procedure (Lang, 1958).

Growth curves on the 2 different culture media used in this study were carried out by haemocytometer counts at regular intervals. Counts on diphasic cultures were carried out on six cultures in duplicate, each point on the growth curve thus representing the average of six measurements in duplicate. Counts on all-liquid cultures were carried out on 12 to 24 separate cultures and each point represents the average value of these counts.

EXPERIMENTAL

Growth. Initial studies were carried out with organisms harvested from diphasic cultures. Neither the harvesting of the organisms and cleaning them of residual culture medium material nor the initial preparation of culture medium could be sufficiently standardized to consider the results completely reproducible from one experiment to another. However, the growth curve obtained under such conditions (fig. 1) agrees in essential features with those reported by von Brand et al (1946) and Chang and Negherbon (1947) who used similar media.

The growth curve in HM medium is shown in figure 1. After it was found to be satisfactory, other experiments were carried out with organisms cultured in the liquid medium.

Cell nitrogen determinations were carried out on washed organisms harvested

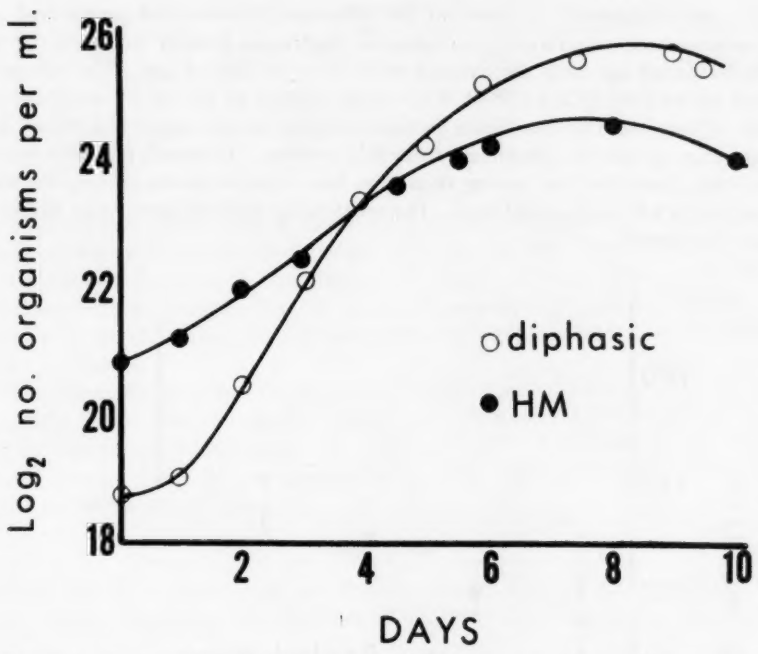

FIGURE 1. Growth curves of *Trypanosoma cruzi* in diphasic medium and HM medium.

from diphasic and HM cultures of different ages. Within the HM group the total nitrogen content of cells from cultures of different ages was found not to vary appreciably. However, the nitrogen content per organism of cells grown on diphasic media was significantly different from that of cells grown on HM. Samples of suspensions used for respiratory measurements were taken for nitrogen analysis. It was found that the mean nitrogen per 10^6 organisms for trypanosomes harvested from diphasic medium and from HM medium was 1.76 micrograms and 1.10 micrograms, respectively. These nitrogen analyses are summarized in table I.

Respiration and Age of Cultures. The respiratory rates of *S. cruzi* at different stages of growth in culture medium were determined with glucose as a test sub-

TABLE I. *Nitrogen content of* T. cruzi *culture forms in diphasic and HM medium.*

Diphasic medium		HM medium	
mg. N/10^{6}* cells	Age of culture in days	mg. N/10^{6}* cells	Age of culture in days
1.57	4	1.03	4
2.15	5	1.25	4
1.37	8	1.01	4
1.43	13	0.87	7
2.28	14	1.01	7
		0.92	7
		1.12	8
		0.92	10
		1.42	11
		1.11	12
		1.40	13
Mean:	1.76 ± 0.17	Mean:	1.10 ± 0.06

* Each figure represents average of duplicate determinations.

strate, and comparison was made of the differences between endogenous and substrate-stimulated respiration. Endogenous respiration showed an increasing rate with increasing age until the cultures were 13 to 14 days of age. The values obtained varied from QO_2 (N)* of 91 for 4-day cultures to 170 for 11- to 12-day cultures. Organisms harvested from diphasic medium showed higher respiratory rates in some age groups than organisms from HM medium. However, the mean respiratory rates determined by pooling data from both types of media overlap the mean rates obtained from unpooled data. The relationship between these mean values are shown in figure 2.

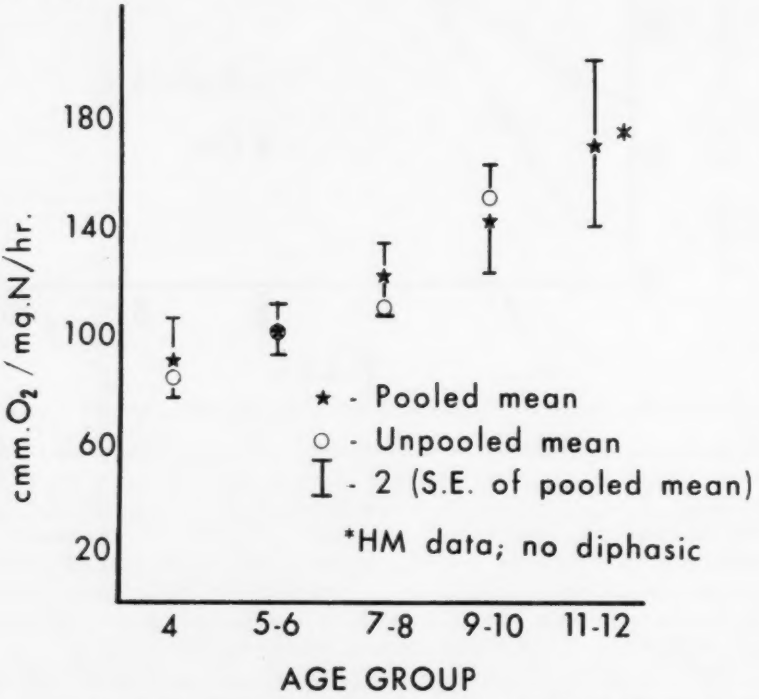

FIGURE 2. Mean endogenous respiratory rates obtained with pooled and unpooled data.

The relative increase in respiration above the endogenous rate effected by glucose remained quite constant through 12 days of cultivation. Organisms from cultures beyond the peak of the growth curve were stimulated less by glucose than those from the preceding age group. The range for culture forms harvested from 4 to 12 days of age had mean values ranging from 146 percent to 169 percent of the endogenous rate, with no change in general trend as cultures aged (table II). This, of course, means that the respiration in the presence of glucose increased with age using cells from 4- to 12-day cultures. A decrease was again noted at 13 to 14 days (fig. 3).

Effect of Concentration of Organisms and of Substrate on Respiration. It was found that endogenous and glucose-stimulated respiration are independent of para-

* QO_2 (N) = microliters of oxygen consumed per mg nitrogen per hour.

TABLE II. *Effect of glucose on respiration of* T. cruzi *culture forms at 32 C.*

Age of culture	Respiration as percent of endogenous	S.E.	Number of samples
4 day	168	27	15
5 to 6 day	164	8	7
7 to 8 day	167	14	16
9 to 10 day	146	7	10
11 to 12 day	169	16	13
13 to 14 day	135	7	9

site concentration in the range of 0.4 to 1.7×10^8 organisms per ml (fig. 4). During the course of the present study many different concentrations of parasites were used in different experiments, ranging from 0.4 to 2.9×10^8 organisms per ml. However, there was no evidence that this variation influenced the results obtained. A scatter diagram of parasite concentration versus endogenous oxygen consumption is shown in figure 5.

The effect of glucose concentration on respiration was examined. The rate was essentially independent of substrate concentration between 7×10^{-3} M and 2.8×10^{-4} M. At concentrations between 2.8×10^{-4} M and 7×10^{-5} M, the rates varied from no evident increase above the endogenous rate to a rate identical with that obtained at higher concentrations of substrate.

DISCUSSION

Since *Schizotrypanum cruzi* was first cultured in vitro by Chagas (1909b) on blood agar medium (Novy and McNeal, 1904), various modifications of the original

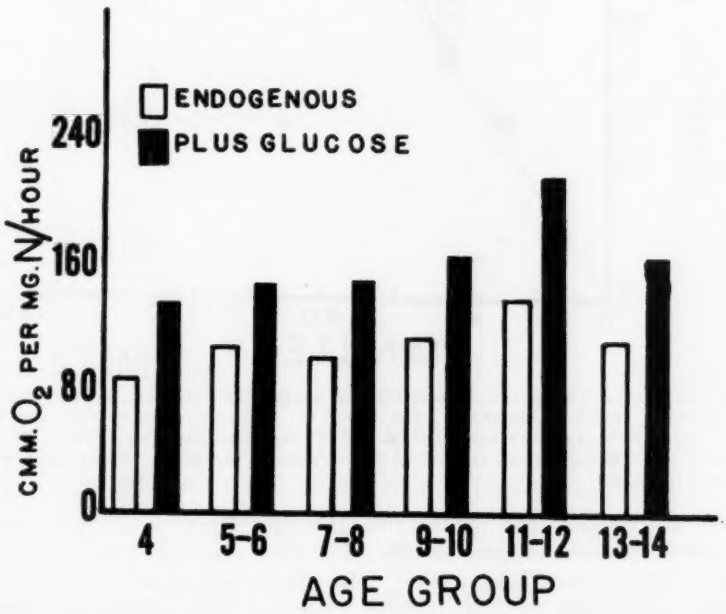

FIGURE 3. Comparison of respiration in the presence and absence of added substrate (glucose) for organisms harvested from cultures 4 to 14 days of age.

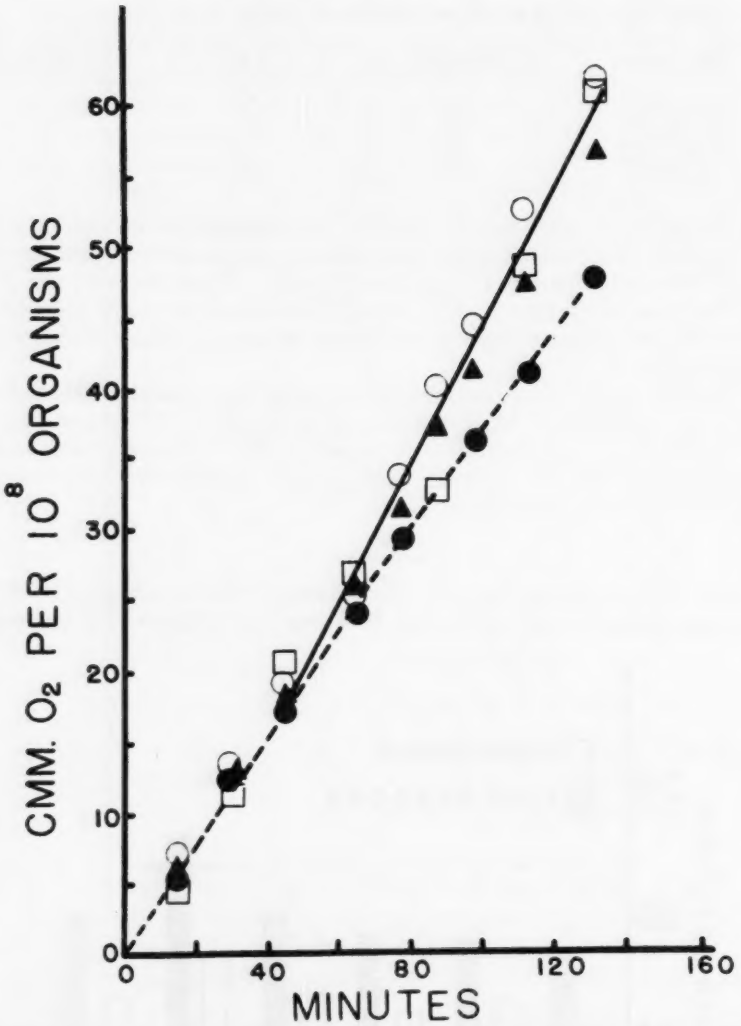

FIGURE 4. Effect of parasite concentration on glucose stimulated and endogenous respiration. Diphasic medium (incoulum: 7-day culture). Substrate concentration: 7.0×10^{-3} M glucose. □ 0.9×10^8 organisms per vessel, ▲ 2.9×10^8 organisms per vessel, ○ 4.7×10^8 organisms per vessel. ● Endogenous control, 1.8×10^8 organisms per vessel. Arrow indicates time when substrate added.

medium have been used for culturing this organism. The most widely used type is the so-called diphasic medium. Media of the latter type have been developed by Senekjie (1943), and Chang (1948). Partially defined liquid media have been described by Sampath and Little (1949), Little and Oleson (1951) and Citri and Grossowicz (1955). The latter workers grew *S. cruzi, T. lewisi, T. acomys, "Herpetomonas culicidarum," Leishmania tropica,* and a *Leishmania sp.* (from reptiles)

in a medium consisting of defined constituents (inorganic salts, glucose, hematin, vitamins, cobalamin, ribonucleic acid, cytidylic acid, creatine and creatinine) plus an enzymatic hydrolysate of casein. *S. cruzi* was grown in this partially defined medium through 11 passages, after having previously grown for 2 years in the same medium plus crystalline serum albumin. This medium is probably the best yet devised for carrying out nutritional studies on *S. cruzi* but, for routine work in which specific growth factors are not being considered, it has disadvantages in being complex, expensive, and containing heat-labile constituents. The results of the present study indicate that the HM medium has the following advantages over other media used for the cultivation of *S. cruzi:* (1) It is liquid and free of particles; (2) it can be autoclaved; (3) it is easily prepared at relatively low cost from a commercially available dehydrated material (Difco brain-heart infusion); (4) defibrinated sheep's blood is commonly available in large quantities; (5) growth can

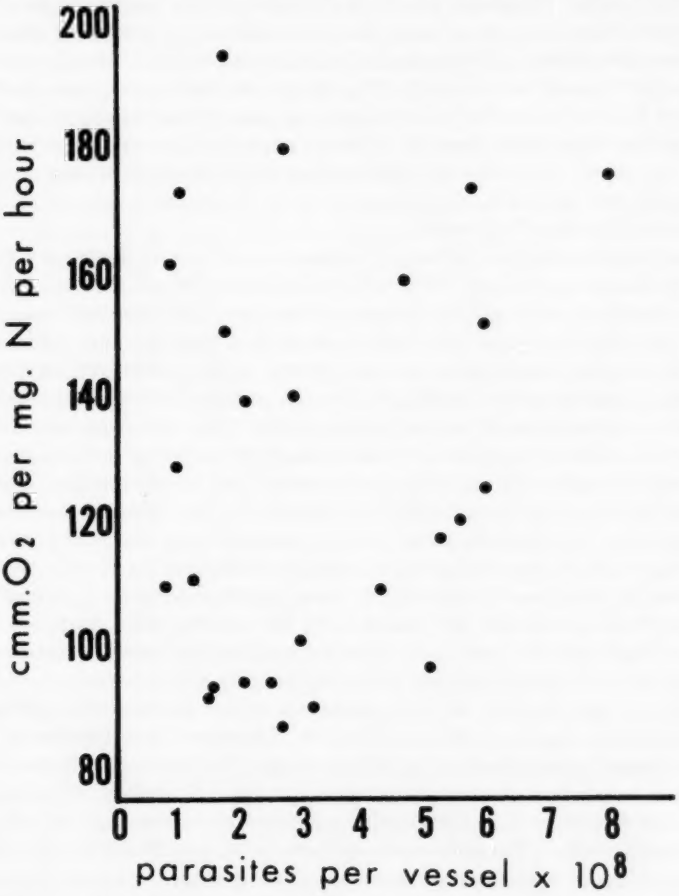

FIGURE 5. Scatter diagram of mean endogenous oxygen consumption versus concentration of parasites. Total volume of suspending medium was 2.6 ml.

be accomplished in large or small volume with equal ease; (6) the total growth attained in the HM medium is relatively high (ca 25 to 35×10^6 organisms per ml); and (7) the rate of growth is about as rapid as that reported for other culture media. The present study indicated that for the different phenomena investigated there was less variability in organisms grown on HM medium than in those grown on diphasic medium. This difference was reflected in the results obtained for nitrogen determinations, growth curves, and respiratory measurements. When the HM medium was compared with the diphasic medium the following differences were noted. First, total growth, (i.e., peak population) was almost twice as high in diphasic medium as in HM medium. However, it may be pointed out that there are practical limitations on the total volume of diphasic medium that can be prepared at one time, whereas large volumes of HM medium can be prepared and stored with ease. Second, the nitrogen content per organism was found to differ in the two media used in the present study. Organisms grown on diphasic medium contained about 50 percent more nitrogen than those grown on HM medium. It was noted that washed organisms from diphasic cultures contain red pigments, whereas those grown on HM medium have a dark brown color. The organisms grown in diphasic medium, in which the blood is subjected to a minimum amount of heat, appear to possess the pigmentation of the blood elements. On the other hand, when grown in HM medium, they appear to possess the pigmentation of the brain-heart infusion medium. The higher and more variable nitrogen content of organisms grown on diphasic medium may be related to this difference.

Most investigators have expressed metabolic rates of protozoans as activity per unit organism, commonly per 10^6 or 10^8 organisms (von Brand, 1952). The present study indicates the difficulty in comparisons on a per unit organism basis. In the work reported herein gross differences in metabolic activity per unit organisms were noted for the same strain grown in two different media. However, after reducing the unit of organisms to a function of nitrogen content the discrepancy in metabolism between cells grown in two media was negated. Our data expressed in terms of nitrogen are in fair agreement with those reported by von Brand and Agosin (1955). These authors reported a value of approximately 1 mg of nitrogen per 10^9 cells for *S. cruzi* (Brazil strain) grown in diphasic medium. This value is in agreement with nitrogen values for organisms grown on HM medium, but it is only 57 percent of the value obtained for organisms grown in diphasic medium in the present study. Recalculation of data given by Ryley for *S. cruzi* (strain undesignated) agrees roughly with the results obtained in the present study for a 14-day-old culture.

Von Brand and his co-workers (1946) found that the rate of respiration of *S. cruzi* in culture decreased with the age of the cultures and a decrease in respiratory quotient was also observed to occur during the decline phase of the growth curve. Von Brand and Agosin (1955) indicated no differences in respiration of washed *S. cruzi* from diphasic cultures 7 to 12 days of age. The present study has shown an increase in respiration with increasing age of cultures. Thus, different investigations have given rise to each of the possible relationships between age of culture and respiratory activity. The early study on cultures by von Brand et al (1946) was carried out under experimental conditions differing from those of von Brand and Agosin (loc. cit.) and the present study. The work reported herein and that of von Brand and Agosin were carried out using very similar techniques. It does not seem

likely that the differences in results obtained were the consequences of differences in method of analysis. The fact that in both cases (von Brand and Agosin and the present study) the results differ from those obtained in the earlier study on *S. cruzi* may indicate a physiological change in the strain after 10 years of passage in vitro.

Moulder (1948), in his study of the oxidative metabolism of *T. lewisi* during its growth cycle in the rat, concluded that the metabolic changes observed were attributable to the appearance of the reproduction-inhibiting antibody (ablastin), but it is also possible that the changes observed were characteristic of the life cycle of *T. lewisi* in its vertebrate host, and similar to the pattern observed in the present work. Thurston (1958) reported a constant rise in respiratory rate of *T. lewisi* for the first 7 days when glutamine was used as a substrate, and then a leveling off at the eighth day of infection.

Some observations have been made on the changes in respiratory rate of *Leishmania donovani* (Fulton and Joyner, 1949). A comparison of the respiration of organisms harvested from 6-, 8- and 10-day-old cultures showed a sharp drop from the 6th to the 8th day, and then a more gradual decrease between the 8th and 10th day; unfortunately, the ratios of 6- to 4-day rates were not given. *Leishmania* cultures undergo degenerative changes much more rapidly than *S. cruzi* (Chang, 1948), and the drop observed in the present study from the 12th to the 14th day may correspond to the 8th to 10th day period of *L. donovani*.

Parasite density has been reported to affect the respiratory rate of *Trypanosoma rhodesiense* when the organisms exceeded 200 million per ml (von Brand and Tobie, 1948). Harvey (1949) found that respiration of *T. hippicum* was affected when the parasite density exceeded 200 million. However, von Brand and Agosin (1955) did not report any effect on respiration of parasite density when parasites varied from 100 to 500 million per ml; the present study yielded similar results.

According to von Brand et al (1949), the culture forms of *S. cruzi* grown in diphasic medium lower the glucose concentration, measured as reducing material, until a concentration of 2.7×10^{-3} M is reached; below this concentration the organism cannot utilize glucose. If glucose utilization is dependent upon substrate concentration, the stimulated respiratory rate should also be dependent upon the concentration of this substrate. The hypothesis of a relationship between change in respiratory rate and lowering of substrate concentration is supported by the observation that pH of cultures progressively declines and then begins to rise, the latter probably occurring after the limiting glucose concentration is reached. It should be indicated here that during growth the pH exceeds the initial pH, probably due to ammonia production. If the increase in pH did not exceed the initial value, the results reported by von Brand et al (1949) could be attributed to the organisms using the end products of their own carbohydrate metabolism (von Brand and Agosin, 1955). Our experiments on the effect of various glucose concentrations do not corroborate the conclusions of von Brand et al (1949) in the following respects: (1) The degree of stimulation of respiration over the endogenous rate was not significantly lowered until a concentration less than 2.8×10^{-4} M was reached; (2) experiments on respiration with glucose at concentrations between 1.4×10^{-4} M and 5.2×10^{-4} M indicate that the quantity of glucose used is proportional to concentration. The results of von Brand et al can probably be explained by the production of non-sugar reducing substances before, but not after, incubation. In contrast with the

present observations on *S. cruzi*, Harvey (1949) found that respiratory rate of *T. hippicum* was dependent upon glucose concentration below 3×10^{-3} M.

SUMMARY

Quantitative differences in organisms grown on brain-heart infusion and diphasic media have been observed: nitrogen content and respiration per unit organism as well as total growth in diphasic medium was significantly higher than that observed in brain-heart infusion medium. However, respiration per unit parasite nitrogen was not significantly different.

The respiratory rate of culture forms of *S. cruzi* in the presence or absence of glucose increases with increasing age of culture. The percent stimulation of respiration by glucose over the endogenous rate remains quite constant throughout the age groups studied.

Endogenous and glucose-stimulated respiration was found to be independent of parasite concentration in the range 0.4 to 2.9×10^8 organisms per ml.

The effect of glucose concentration on the respiratory rate of *S. cruzi* culture forms was examined in the range 7×10^{-5} M to 7×10^{-3} M.

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RESEARCH NOTE

OVERWINTERING OF HELMINTHS IN HIBERNATING ANIMALS.

In cold climates hibernating mammals undergo a profound drop in body temperature, the minimum temperatures being just above the freezing point of water. Blanchard (1903, Compt. Rend. Soc. Biol. **55:** 1120–1126) reported failure to find intestinal worms in hibernating marmots in Europe. Blanchard and Blatin (1907, Arch. Parasit. **11:** 361–378) also reported failure to find any intestinal helminths in 100 hibernating marmots.

Simitch and Petrovitch (1954, Riv. Parassit. **15:** 655–662) reported that the intestinal protozoa of the European ground squirrel, *Citellus citellus*, remain in the host without regard to the level of temperature depression or the duration of hibernation, while various species of naturally occurring intestinal helminths were eliminated from the host after 10 to 20 or more days of uninterrupted hibernation. Since the ground squirrel does not undergo long periods of uninterrupted hibernation, many of its intestinal worms overwinter in the host.

The present author thought it strange that none of the helminths of the marmot had adapted to survive the host's hibernation period in the adult stage. In the spring of 1959, 13 woodchucks (*Marmota monax*) were shot in late April and early May in central Vermont. It is doubtful if any of these animals had been above ground for more than a week. This is not to say that the period of hibernation may not have ended earlier. Examination of the small intestines of these animals revealed viable helminths in 7 of the 13. All positive hosts contained from 4 to 50 bursate nematodes, and 2 also yielded a few other nematodes. Since all these worms appeared to be adults and the hosts would have had almost no chance of acquiring infection so early in the spring, it is suggested that these parasites were successful in overwintering. It is impossible to tell the exact periods of hibernation in these animals or the periods of uninterrupted hibernation within the hibernation period. In this area the woodchucks usually remain underground for at least 5 months.—ROBERT M. CHUTE, *San Fernando Valley State College, Northridge, California.*

RESEARCH NOTE

ANOPHELES FREEBORNI AND *A. QUADRIMACULATUS* AS EXPERIMENTAL VECTORS OF *PLASMODIUM CYNOMOLGI* AND *P. INUI.*

As a part of the work on exoerythrocytic stages of primate malarias, it was necessary to select vector mosquitoes which would be satisfactory for producing heavy sporozoite infections. To this end, a comparison was made of the efficiency of laboratory strains of *Anopheles quadrimaculatus* and *A. freeborni.* The former was already known to support infection with *P. cynomolgi* (Coggeshall, 1941, Am. J. Trop. Med. **21:** 525–530; also, personal communication from Dr. L. H. Schmidt), but unsatisfactory infections were frequently obtained. No information could be found on *A. freeborni.*

To compare the susceptibility of the 2 *Anopheles* to *P. cynomolgi* infection, 9 experiments were conducted in which lots of the 2 species were fed consecutively on *Macaca mulata* monkeys with blood-induced malaria infections. Cages containing up to several hundred unfed, hungry, 4- to 6-day-old mosquitoes were applied to the abdomen of the monkeys. Engorged mosquitoes were removed from these cages and placed in smaller cages where they were kept until dissection. Only fully engorged mosquitoes were selected. The *A. quadrimaculatus* averaged somewhat larger than the *A. freeborni,* and presumably took proportionately more blood. The first 4 trials utilized a strain of *P. cynomolgi* obtained from Dr. L. H. Schmidt of the Christ Hospital, Cincinnati, Ohio, and the last 5 a recently isolated strain obtained from Professor P. C. C. Garnham of the London School of Hygiene and Tropical Medicine. This strain has been named *P. cynomolgi* subspecies *bastianellii* (Garnham, 1959, Riv. Parassit. **20:** 273–278).

Gut dissection was done 5 to 9 days after feeding, and the comparative evaluation was based on proportions infected and actual counts of the oocysts. The results are summarized in the following table:

Trial No.	Monkey No.	*Anopheles freeborni*			*Anopheles quadrimaculatus*		
		No. dissected	Percent positive	Mean oocysts per gut	No. dissected	Percent positive	Mean oocysts per gut
1	P44	18	28	0.9	23	4	0.1
2	P16	10	40	25.3	17	59	4.6
3	P16	14	93	32.9	20	15	0.2
4	P16	19	58	33.5	22	27	3.2
5	603	6	67	13.5	16	81	12.7
6	603	4	100	113.5	4	25	2.3
7	632	10	90	131.8	10	70	27.0
8	575	9	33	8.8	11	45	1.0
9	632	10	70	71.1	10	80	5.0
All		100	60	—	133	41	—

In each instance the oocyst number was higher in *A. freeborni* and in the majority of instances the percentage infected was also greater.

The results of the experiments show quite conclusively that *A. freeborni* is the superior vector so far as intensity of infection is concerned. In other experiments good infections averaging in some instances 1 to 200 cysts per gut were obtained. Sporozoite infections have appeared to be proportional to the cyst number, and transmission has been accomplished with invariable success in more than 20 instances either by bite or by the injection of infected glands. Sporozoite numbers have been sufficient to establish patent liver infections with preerythrocytic forms of *P. cynomolgi.*

Although comparative feedings were not done, fair oocyst infections of *Plasmodium inui* in *Anopheles freeborni* resulted in 3 instances. All feedings were on a single monkey which had not been splenectomized. This is the first demonstration that this *Anopheles* species is susceptible to this parasite. Due to the small number of mosquitoes available, it was impractical to search for sporozoites or attempt transmission.

In later work *A. quadrimaculatus* was fed serially on 2 *P. inui* infections in splenectomized monkeys. Only very scant infections were obtained in spite of very high parasite counts. In spite of the poor infections, *P. inui* was transmitted by injecting dissected glands into a clean monkey intravenously.

In spite of the apparent superiority of *A. freeborni* as a vector of *P. cynomolgi,* and possibly *P. inui,* I have also used *A. quadrimaculatus* in my work on exoerythrocytic parasites. It exhibits superior longevity under insectary conditions and is sometimes easier to rear.—Don E. Eyles, *Laboratory of Parasite Chemotherapy, NIAID, NIH, 874 Union Avenue, Memphis 3, Tennessee.*

COMPARATIVE PHYSIOLOGICAL STUDIES ON FOUR SPECIES OF HEMOFLAGELLATES IN CULTURE. II. EFFECT OF CARBOHYDRATES AND RELATED SUBSTANCES AND SOME AMINO COMPOUNDS ON THE RESPIRATION*

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Reports on the quantitative effect of carbohydrates and other potential energy sources on the respiration of hemoflagellates, especially from a comparative standpoint, are rather scanty. Colas-Belcour and Lwoff (1925), Noguchi (1926) and Kligler (1926) reported that some of the members of the group can ferment certain sugars in complex culture media. More recently, Chang (1948) using manometric techniques, reported the utilization of glucose and fructose but not maltose or lactose by *Schizotrypanum cruzi* and three species of *Leishmania*. Fulton and Joyner (1949) studied the oxidation of several carbohydrates by the flagellate form of *L. donovani* and Medina et al (1955) described similar studies with *L. brasiliensis*. The effect of certain carbohydrates and some amino compounds on the respiration of *Trypanosoma lewisi* and a form termed *Strigomonas oncopelti* have also been observed (Ryley, 1951, 1955). Thurston (1958) studied the oxygen uptake of *T. lewisi* and *T. equiperdum* in the presence of amino acids and similar substances. Finally, Chatterjee and Ghosh (1959) repeated part of the work done by Fulton and Joyner (loc. cit.) with the kala-azar organism.

In this second report, data are presented on the effect of a series of carbohydrates and related substances and a few nitrogenous compounds on the respiration of the culture form of four species of hemoflagellates. Appreciation is expressed to Dr. Clark P. Read for his interest in the work and kind advice and criticism throughout the investigation.

MATERIALS AND METHODS

Hemoflagellates. Details of cultivation of the four species used, *S. cruzi, S. vespertilionis, Endotrypanum schaudinni* and *L. enriettii*, have been given in a separate paper (Zeledón, 1960 a).

Manometric experiments. Treatment of the parasites for manometric experiments and oxygen uptake determinations were carried out as already described (Zeledón, loc. cit.). Duplicate flasks were employed with each substrate which were at a final concentration of 0.01 M. As an exception, the polysaccharides were used at a final concentration of 0.35 percent. Gluconic and glucuronic acids were used in the lactone forms and were dissolved in 0.1 N NaOH, as was ascorbic acid. A correction was made for autooxidation of ascorbic acid by running a control vessel without the flagellates. In the case of nitrogen-containing substrates, 0.2 ml of 3 N HCl were placed in the second side arm of the flask to absorb any ammonia produced. In the latter experiments the reaction volume was kept constant and new values were calculated for the constants of the flasks. The acid substrates were neutralized with NaOH whereas glucosamine hydrochloride and the other amino compounds were dissolved in water. Control vessels containing glucose were included in each experiment and endogenous respiration was also recorded in

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two control flasks. Experiments yielding values which were very close to those of the endogenous respiration were repeated, again in duplicate. All the solutions of substrates used in manometric experiments were stored in the freezer.

Nitrogen determinations. These were carried out either by the technique of Kabat and Mayer (1948) or by the nesslerization procedure described by Lang (1958) using the same standard solution of ammonium sulfate for both techniques.

RESULTS

Qualitative and quantitative differences were noticed in the ability of several carbohydrates and related substances to stimulate the endogenous respiration of the four species of hemoflagellates (table I). Among the monosaccharides, fructose and mannose stimulated the respiration of the four species to the same extent as glucose. Galactose gave approximately 50 to 80 percent of the increase observed with glucose. Rhamnose increased slightly the endogenous respiration of all the species except *E. schaudinni*. Among the disaccharides, maltose and its isomer cellobiose stimulated respiration; the first to an extent approximately 50 percent that produced by glucose. Cellobiose produced only a slight increase in the respiration of the four species. Sucrose was utilized at a considerable rate by *Endotrypanum* and the *Leishmania* but not by *Schizotrypanum*, and melibiose, an isomer of lactose, was oxidized at an almost negligible rate by the two species of *Schizotrypanum* and by *L. enriettii*. A trisaccharide, raffinose, produced a moderate stimulation of *E. schaudinni* and about the same stimulation as glucose in *L. enriettii*.

Some sugar derivatives, such as sorbitol, alpha-methylglucoside and 2-desoxyglucose showed some stimulation of one or more species but, in general, to a slight extent. Since this type of stimulation, with values very close to endogenous, was reproducible, the differences are considered significant. The results with 2-desoxyglucose are rather interesting since the compound, usually considered as an inhibitor of glycolysis, was utilized by the *Schizotrypanum*, was indifferent in the case of *Leishmania* and depressed the endogenous respiration of *E. schaudinni*. Glycerol, the last substrate in this series, produced a pronounced stimulation of *E. schaudinni*, a low stimulation of respiration of *Schizotrypanum* and was moderately oxidized by *Leishmania* (figs. 1 to 4).

The following substances were without effect upon the endogenous respiration of the four species of parasites: trehalose, lactose, glycogen, starch, ascorbic acid, sodium gluconate and sodium glucuronate.

Experiments on the effect of a few nitrogenous substances on the respiration of the four species (table II) indicated that glucosamine, a compound more closely related to glucose than the others, was utilized at the same rate as glucose by the two *Schizotrypanum*, and at rates corresponding to 78 and 53 percent of the rate of utilization of glucose, by *L. enriettii* and *E. schaudinni* respectively. Glutamine, asparagine and glutamic acid were used by all of them except *L. enriettii*. Aspartic acid was used only by *E. schaudinni* and *S. cruzi*. Alanine gave a moderate stimulation in the case of *Endotrypanum*, representing 61 percent of the increase produced by glucose. Glutamine produced a 15 percent higher stimulation of respiration of *S. cruzi* than did glucose (figs. 5 and 6).

DISCUSSION AND CONCLUSIONS

The utilization of sugars other than glucose has been reported in several species and there is general agreement that, among the monosaccharides, fructose and man-

TABLE I. *Effect of some carbohydrates and related substances on the respiration of the four species of hemoflagellates. Each figure represents the mean of one duplicate determination, unless otherwise stated.*

Species		Endogenous	Glucose	Fructose	Galactose	Mannose	Rhamnose	Maltose	Cellobiose	Saccharose	Melibiose	Raffinose	Sorbitol	alpha-methyl-glucoside	2-desoxyglucose	Glycerol
E. schaudinni	S/E	1.00	13.80*	12.53	6.50	11.88	1.00	8.79**	1.45	13.60	1.00	3.47	1.44**	2.18**	0.69**	13.10
	S/G	0.09*	1.00	0.97	0.50	0.92	0.11	0.59**	0.18	0.94	0.11	0.24	0.08**	0.12**	0.12**	0.90
L. enriettii	S/E	1.00	7.18*	5.90	4.18	7.81	1.48**	2.46	1.52	6.00	1.27**	7.81	1.00	1.09	1.00	4.09
	S/G	0.14*	1.00	1.00	0.50	0.95	0.23**	0.42	0.21	0.98	0.20**	0.95	0.16	0.17	0.14	0.67
S. vespertilionis	S/E	1.00	3.04*	4.64	3.91	4.41	1.59**	2.38	1.65**	1.00	1.33**	1.00	1.08	1.05	2.01**	1.60**
	S/G	0.36*	1.00	1.00	0.83	0.94	0.36**	0.67	0.36**	0.23	0.28**	0.12	0.19	0.25	0.44**	0.46**
S. cruzi	S/E	1.00	2.78*	3.89	2.89	3.68	1.18**	2.64**	1.29**	1.00	1.16**	1.04	1.00	1.04	1.36**	1.51**
	S/G	0.39*	1.00	1.00	0.74	0.94	0.37**	0.59**	0.40**	0.29	0.36**	0.32	0.31	0.30	0.40**	0.49**

S/E = ratio Q^N_{O2} substrate/Q^N_{O2} endogenous ; S/G = ratio Q^N_{O2} substrate/Q^N_{O2} glucose ; Q^N_{O2} = µl of O_2/mg N/hr.
* Mean of 15 duplicate determinations. ** Mean of 2 duplicate determinations.

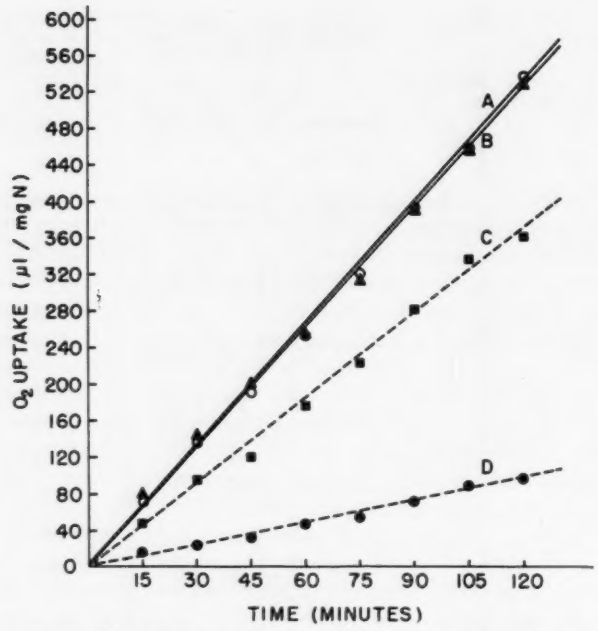

FIGURE 1. Effect of carbohydrates and related substances on the respiration of *L. enriettii*. A, glucose, B, saccharose, C, glycerol, D, endogenous.

nose produce the same stimulation as glucose, and galactose is oxidized with more difficulty (cf. von Brand, 1952; Chang, 1948; Fulton and Joyner, 1949; Ryley, 1951, 1955). Maltose seems to be a disaccharide which is frequently utilized although at a relatively low rate (cf. von Brand, 1951). Chang (1948) reported that the culture forms of *S. cruzi, L. brasiliensis, L. tropica* and *L. donovani* do not utilize maltose.

The oxidation of sucrose at a rate practically identical with that of glucose by *E. schaudinni* and *L. enriettii* is rather interesting. Further study is necessary to de-

TABLE II. *Effect of some nitrogenous substances on the respiration of the four species of hemoflagellates. Each figure represents the mean of one duplicate determination, unless otherwise stated. Glucose and endogenous values are given for comparison.*

Species		Endogenous	Glucose	Glucosamine	Asparagine	Glutamine	Glutamic acid	Aspartic acid	Alanine
E. schaudinni	S/E	1.00	13.80*	6.53	1.56**	5.28	2.08	1.43**	7.50
	S/G	0.09*	1.00	0.53	0.17**	0.79	0.31	0.11**	0.61
L. enriettii	S/E	1.00	7.18*	7.52	0.90	1.00	1.00	1.00	0.84
	S/G	0.14*	1.00	0.78	0.11	0.13	0.13	0.16	0.09
S. vespertilionis	S/E	1.00	3.04*	1.98	1.66	2.59	1.96	1.07	1.02
	S/G	0.36*	1.00	0.97	0.65	1.02	0.77	0.52	0.50
S. cruzi	S/E	1.00	2.78*	2.08	1.54	2.45	1.89	1.23**	1.02
	S/G	0.39*	1.00	0.95	0.72	1.15	0.88	0.58**	0.47

S/E and S/G ratios as in Table I.
* Mean of 15 duplicate determinations. ** Mean of 2 duplicate determinations.

termine whether hydrolysis is involved in sucrose degradation or the dissimilation involves direct phosphorylation as in certain other microorganisms (Doudoroff et al, 1943) or some other mechanism. Fulton and Joyner (1949) were unable to demonstrate any stimulation by sucrose, over the endogenous respiration, in the culture form of *L. donovani*. On the other hand, Chatterjee and Ghosh (1958) reported some kinetic studies on a sucrose-splitting enzyme in cell-free extracts of the same organism. The Indian authors were able to demonstrate that the enzyme is purely hydrolytic in nature, splitting the disaccharide into a mixture of free glucose and free fructose. More recently, Chatterjee and Ghosh (1959) have shown that sucrose is oxidized at practically the same rate as glucose by intact cells of the causative organism of kala-azar.

The trisaccharide raffinose, a common plant sugar, could be hydrolyzed by the sucrose-splitting enzyme or even by maltase in the case of *L. enriettii* and *E. schaudinni*. If hydrolysis occur, it would appear to be less effective in *Endotrypanum* and its products would be available only in limited amounts. Whether or not other carbohydrates besides glucose are utilized by the four species, seems to be correlated with the apparent relative importance of carbohydrate metabolism in each particular case. Furthermore, the dissimilation of glycerol at different rates in the organisms is further confirmation of the above statement indicating, for example, that glucose is oxidized very effectively by *E. schaudinni*, which depends on this type of substrate, whereas quite a different picture is presented by *Schizotrypanum*. Oxidation of

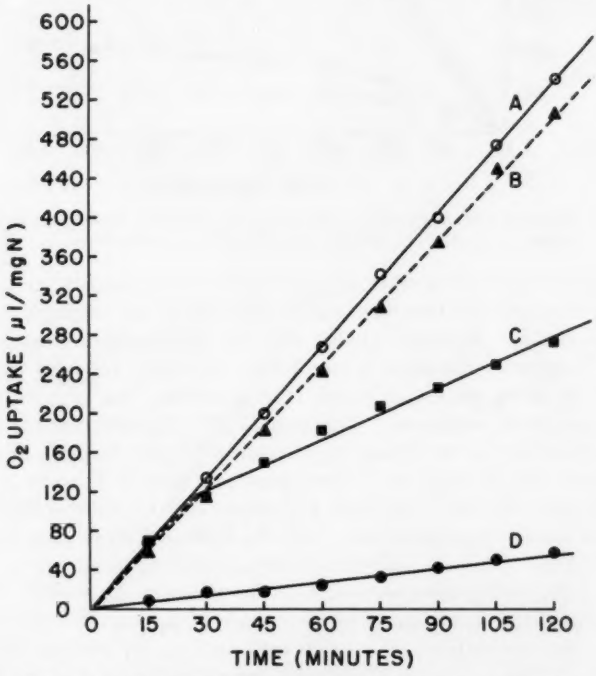

FIGURE 2. Effect of carbohydrates and related substances on the respiration of *L. enriettii*. A, glucose, B, raffinose, C, galactose, D, endogenous.

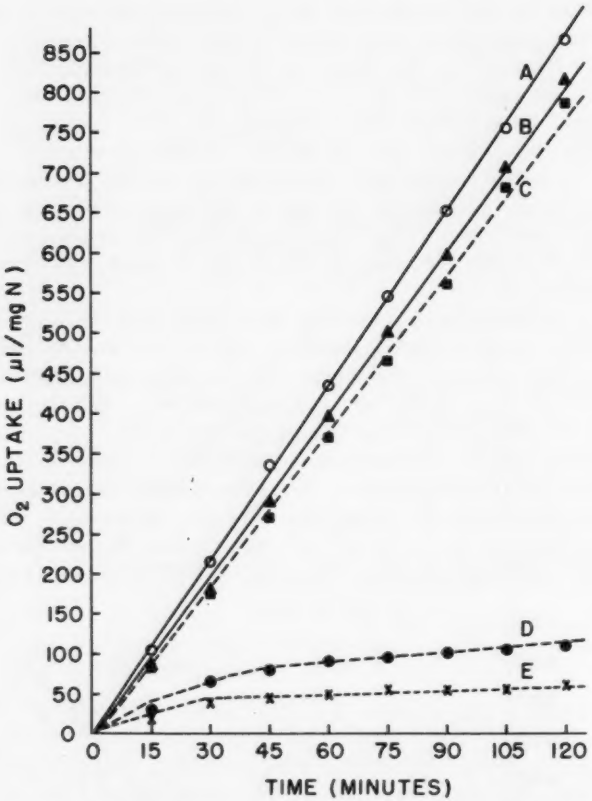

FIGURE 3. Effect of carbohydrates and related substances on the respiration of *E. schaudinni*. A, glucose, B, sucrose, C, glycerol, D, alpha-methylglucoside, E, endogenous.

glycerol has been reported in other trypanosomids, and it seems to be used at high rate by those trypanosomes that show marked dependence on a suitable carbohydrate substrate for vitality. Marshall (1948) reported that glycerol is used as well as glucose by *T. evansi*. The same is true for *T. hippicum*, regarded by some as a synonym of the latter species, in which Harvey (1949) was able to demonstrate phosphorilation of the compound. Thurston (1958) reported that the oxygen uptake by *T. equiperdum* in the presence of glycerol is slightly lower than in the presence of glucose. On the other hand, the bloodstream form of *T. lewisi* oxidizes glucose at about twice the rate of glycerol (Moulder, 1948). Ryley (1951), working with the same species, reported a rate of glucose oxidation about three times that of glycerol oxidation. With *S. oncopelti* Ryley (1955) obtained up to 50 percent stimulation of the endogenous respiration in the presence of glycerol. The report by Baernstein (1953) of the inactivity or absence of alpha-glycerophosphate dehydrogenase in the culture form of *S. cruzi* suggests that the enzyme plays a minor metabolic role. This is consistent with the present observation of the low rate of utilization of glycerol by this species.

The behavior of the four species in relation to the compound 2-desoxyglucose deserves special comment. It is known that this sugar derivative is phosphorylated by hexokinase from several sources but it is not glycolysed. For that reason the compound has been employed as an antimetabolite of glucose in studies with normal and tumor cells which depend to a large extent on glycolysis (Woodward and Hudson, 1954). On the other hand, Williams and Eagon (1959) working with a cell-free extract of *Pseudomonas aeruginosa,* found no inhibition of glucose metabolism by 2-desoxyglucose and attributed it to the fact that the hexosemonophosphate scheme is the major pathway of the metabolism of glucose in the bacterium. The compound was oxidized to 2-desoxygluconic acid without phosphorylation. This seems to be the first record of dissimilation of this sugar derivative. Thus, the oxidation of 2-desoxyglucose by the two species of *Schizotrypanum* deserves further investigation, since no pathway of glucose dissimilation other than the Embden-Meyerhof-Parnas sequence has been demonstrated in Trypanosomidae.

The oxidation of glucosamine at a different rate than glucose by *Endotrypanum* and *Leishmania* might indicate different affinities for this compound on the part of hexokinase, with different rates of phosphorylation. Chatterjee et al (1958) reported that hexokinase from *L. donovani* has a very marked action upon glucose,

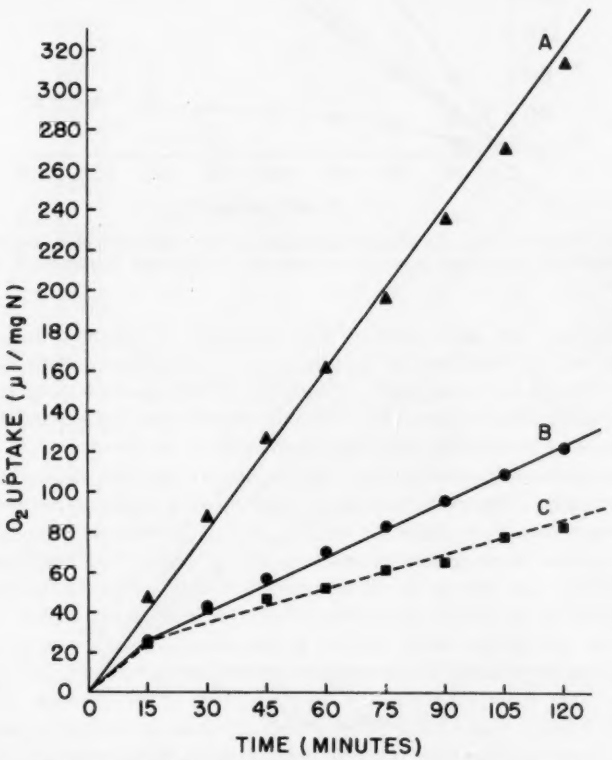

FIGURE 4. Effect of carbohydrates and related substances on the respiration of *S. cruzi.* A, glucose, B, 2-desoxyglucose, C, endogenous.

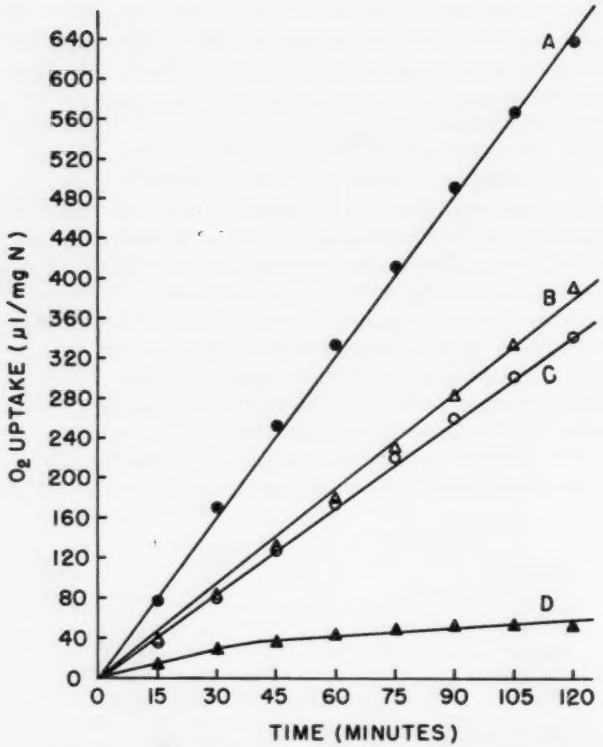

FIGURE 5. Effect of some nitrogenous substances on the respiration of *E. schaudinni*. Glucose and endogenous patterns are given for comparison. A, glucose, B, alanine, C, glucosamine, D, endogenous.

mannose, fructose and glucosamine. The possibility of a specific hexokinase for glucosamine in hemoflagellates, of the type found in *Schistosoma mansoni* (Bueding et al, 1954) should be investigated. Thurston (1958) reported a vigorous oxygen uptake by the bloodstream form of *T. lewisi* in the presence of glucosamine while "no oxygen uptake was recorded" with this substance by *T. equiperdum*.

Another substance promoting high rates of oxygen uptake in several hemoflagellates is glutamine. Organisms that do not depend very much on the presence of a suitable exogenous carbohydrate for vitality, such as the two species of *Schizotrypanum*, can oxidize the compound at a rate equal to or higher than that observed with glucose. This is also true of the bloodstream form of *T. lewisi* (Thurston, 1958), and of the cultural form of *L. donovani* (Chatterjee and Ghosh, 1959). It has also been reported that glutamine is oxidized by the plant parasite *S. oncopelti* (Ryley, 1955). On the other hand, *T. equiperdum*, which dies immediately after removal of the carbohydrate, does not show any oxygen uptake in the presence of glutamine (Thurston, 1958). Some kind of intermediate situation seems to prevail in *E. schaudinni* since this organism is highly dependent on the presence of carbohydrate for the maintenance of motility and still can oxidize glutamine at a fairly good rate.

Glutamine is very likely hydrolysed directly by the organisms; Ryley (1951) and Thurston (1958) have demonstrated the liberation of ammonia during the oxidation of the compound by *T. lewisi.*

Asparagine, glutamate and aspartate increase the respiration of *T. lewisi* (Moulder, 1948; Ryley, 1951; Thurston, 1958) and of *L. donovani* (Chatterjee and Ghosh, 1959). These compounds and also alanine produced different degrees of stimulation with *S. oncopelti* but, in all cases, less than that produced by glucose (Ryley, 1955). None of these nitrogenous substances have an effect upon the respiration of *T. equiperdum* (Thurston, 1958). Tobie et al (1950) reported that *T. gambiense* and *T. rhodesiense* do not produce ammonia in culture but that this substance accumulates in *S. cruzi* cultures. The results reported by Mannozzi-Torini (1940) with *T. evansi,* are difficult to interpret since all the amino compounds were tested in the presence of glucose. Warren (1957) reported no change in the respiration of *S. cruzi* after addition of alanine, glutamate and aspartate. In the present study the last two substances have been shown to be oxidized by *S. cruzi* though not at a high rate. A strain difference, or the higher endogenous respiration reported by

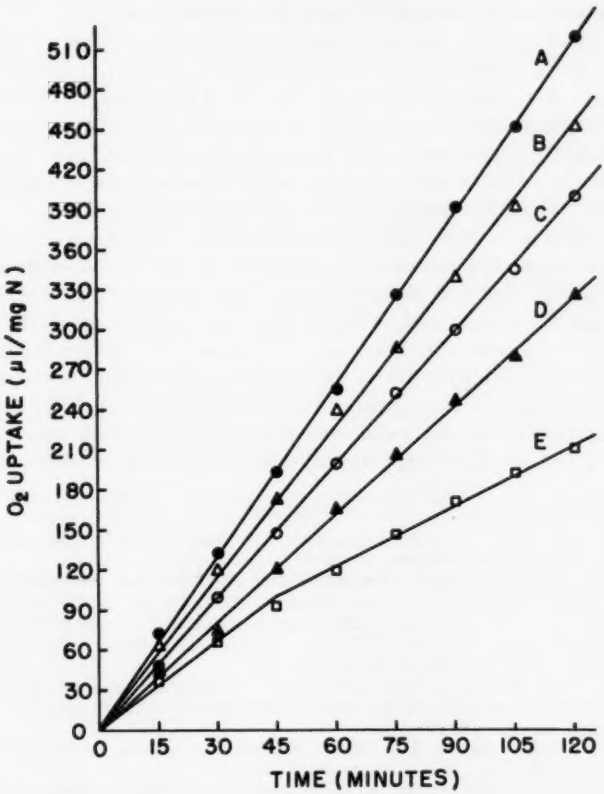

FIGURE 6. Effect of some nitrogenous substances on the respiration of *S. cruzi.* Glucose and endogenous patterns are given for comparison. A, glutamine, B, glucose, C, glutamic acid, D, asparagine, E. endogenous.

Warren (loc. cit.), which would mask the oxidation of the compounds, could account for the noted differences. In the present author's opinion, the oxidation of some amino compounds like alanine, aspartate, glutamate and perhaps asparagine and glutamine, in organisms with a tricarboxylic acid cycle, is probably connected in some way with transaminating enzyme systems, before their utilization as energy sources. The oxidation of alanine at a high rate by *Endotrypanum* together with a particularly active system of transaminases for this particular amino acid (Zeledón, 1959), provides a good example of a nitrogenous compound probably used as energy source by its oxidation through the tricarboxylic acid cycle, which is very active in this species (Zeledón, 1960b).

SUMMARY

A comparison of the capacity of the culture forms of *Endotrypanum schaudinni, Leishmania enriettii, Schizotrypanum cruzi* and *S. vespertilionis* to oxidize several carbohydrates and related substances and a few amino compounds has been made by manometric techniques. The study has revealed significant qualitative and quantitative differences among the three genera represented. A striking physiological similarity was observed between *S. cruzi* and *S. vespertilionis.*

Fructose and mannose gave a respiratory stimulation as great as glucose for all the species; so did sucrose for *E. schaudinni* and *L. enriettii,* and raffinose for the latter species. The trisaccharide was oxidized at a lower rate by *E. schaudinni* as were galactose and maltose by the four species. Glycerol was oxidized almost as well as glucose by *E. schaudinni,* at a lower rate by *L. enriettii* and showed only a slight stimulation of the respiration of the *Schizotrypanum.* Other sugars or related substances with slight stimulatory effect in all or some of the species were: cellobiose, melibiose, rhamnose, sorbitol, alpha-methylglucoside. The sugar analogue, 2-desoxyglucose, inhibited the respiration of *E. schaudinni,* did not affect endogenous respiration of *L. enriettii,* and was utilized at a low but steady rate by *Schizotrypanum.* The following substances were without effect on respiration: trehalose, lactose, glycogen, starch, ascorbic acid, gluconate and glucuronate. *L. enriettii* was the only species incapable of oxidizing any of the nitrogenous substances tested with the exception of glucosamine. This compound was oxidized at rates different from those of glucose by the four species. Glutamine, asparagine, glutamic and aspartic acids and alanine gave moderate to high stimulations of respiration of one or more species.

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THE EFFECT OF ALLOXAN DIABETES ON *PLASMODIUM BERGHEI* INFECTION IN ALBINO RATS*

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Studies on the physiology of erythrocytic stages of malaria parasites indicate that the primary source of energy for their metabolism is glucose which is obtained from the blood of the vertebrate host (Bass and Johns, 1912 and 1913; Christophers and Fulton, 1938 and 1939; Fulton, 1939; and others). Christophers and Fulton (1938) and Fulton (1939) found that an increasing level of parasitemia was accompanied by decreasing levels of blood sugar in monkeys infected with *Plasmodium knowlesi*. Mercado (1952) reported similar findings in albino rats infected with *P. berghei*.

Bass and Johns (1912) reported that the addition of glucose was necessary for successful in vitro cultivation of the human malaria parasites, *P. vivax* and *P. falciparum*. However, blood from a diabetic person who also had malaria was successfully used in the culture medium without addition of glucose (Bass and Johns, 1913). They reported that the amount of quinine sufficient to control similar malaria infections was ineffective in this case. As a result of these observations they came to the conclusion that the elevated blood sugar provided a medium which could better support the growth and reproduction of parasites than could the blood of a non-diabetic person.

Hegner and MacDougall (1926) and MacDougall (1927) produced hyperglycemia in canaries by injecting glucose solutions intraperitoneally or by feeding massive amounts of glucose. In these canaries with high levels of blood sugar, the infection with *P. cathemerium* was much more severe than it was in untreated birds.

Other than the work of Bass and Johns (1913) no studies have been made of the relationship of malaria infections to the hyperglycemic condition produced by diabetes. This is due largely to the difficulty of obtaining human subjects for experimentation and the expense involved in using simian hosts.

The discovery of *Plasmodium berghei* (Vincke and Lips, 1948), a malaria parasite of the rat, and the discovery that alloxan will produce diabetes in the rat (Dunn et al, 1943) have made it possible to study the effect of elevated blood sugar on the course of *P. berghei* infections in diabetic rats. This study was undertaken to determine whether the course of infection of *P. berghei* in diabetic rats is similar to that observed in hyperglycemic canaries infected with *P. cathemerium* (MacDougall, 1927).

MATERIALS AND METHODS

The strain of *P. berghei* used in this investigation was originally isolated by Vincke and Lips (1948) and designated the Kisanga strain. It was obtained through the kindness of Drs.

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Male and female albino rats (*Rattus rattus norvegicus*) of the Sprague-Dawley and Wistar strains were used as the experimental hosts. The animals weighed 87 to 301 g, and were maintained on Purina Laboratory chow and tap water given ad libitum.

To produce diabetes, an aqueous solution of 5 percent alloxan monohydrate (E. H. Sargent) was given intraperitoneally in doses of 80 to 180 mg per kg of body weight. Immediately after the administration of alloxan, a paralysis of the posterior part of the body was noted. Alloxan is chemically related to the barbiturates and may show similar anesthetic effects. Barbiturate anesthesia has been shown to increase sensitivity to alloxan treatment (Kennedy and Lukens, 1944; Shipley and Beyer, 1947).

In a typical experiment, animals were weighed, anesthetized with Nembutal (pentobarbital sodium, Abbott) at doses of 30 to 40 mg per kg of body weight, and inoculated intracardially with blood containing *P. berghei*. The inoculum consisted of infected blood diluted approximately 1 : 100 with 27 mg per 100 ml heparin in 0.85 percent saline. The day of inoculation was considered day zero of the infection. Blood for erythrocyte counts and parasite counts was obtained by clipping off the end of the rat's tail. A sample of blood was drawn accurately into an erythrocyte diluting pipette, diluted 1 : 200 with Hayem's solution, and the erythrocytes counted in a Spencer Bright Line hemocytometer. Parasite counts were made from blood films stained with Giemsa's stain. The degree of parasitemia was expressed as the number of parasites per 10,000 erythrocytes since multiple infections in a single erythrocyte occurred frequently. The probable error of the counts was less than 15 percent (Gingrich, 1932).

Blood sugar determinations were made on whole blood by the method of Folin and Malmros (1929), a micro-method requiring only 0.1 ml of blood. Blood samples were obtained by cardiac puncture from animals under Nembutal anesthesia. Tests were read on a Klett-Summerson photoelectric colorimeter using a No. 42 filter. A known concentration of glucose was included to suggest the accuracy of each series.

RESULTS

The course of infection of P. berghei *in untreated rats.* Ten rats weighing from 87 to 301 g were inoculated with appropriate amounts of blood containing *P. berghei* and the infection followed by daily counts until the host animal either recovered or died. Three distinct types of infection were observed (fig. 2). Seven of the 10 rats underwent severe infections with a rapid proliferation of parasites and eventual death of the rat (fig. 1A). The second kind of infection was seen in 2 rats, weighing in excess of 250 g. Both these animals possessed a strong innate immunity to infection with *P. berghei* as indicated by the course of infection. In 1 of these rats the greatest population of parasites was 600 parasites per 10,000 erythrocytes, and in the other it was 1290. The third type of infection, seen only in 1 rat was characterized by a rapid reproduction of parasites until a peak of 8,600 parasites per 10,000 erythrocytes was reached. Rather than a continued increase in numbers, a crisis occurred followed by a rapid diminution in numbers of parasites until the infection became latent.

The mean number of erythrocytes on day zero was 6.711 ± 0.275 million per cubic mm. As the infection progressed, there was a gradual decline in erythrocytes until at the peak of parasitemia the mean erythrocyte count was 2.403 ± 0.416 million per cubic mm (table I). The extent of red cell destruction in individual rats was

TABLE I. *A comparison of diabetic and non-diabetic rats with respect to infection, erythrocyte counts, and blood sugar levels.*

Exp. no.	Weight of animals	RBC per cu mm		Parasites per 10,000 at peak	Blood sugar			
		Initial	*Peak*		Initial	After allox.	During infection	After inf.
1	213	6719	2403	10,795	101		80	—
2	177	6307	2291	9334	105	104	89	—
3	214	6375		2580	100	341	160	311

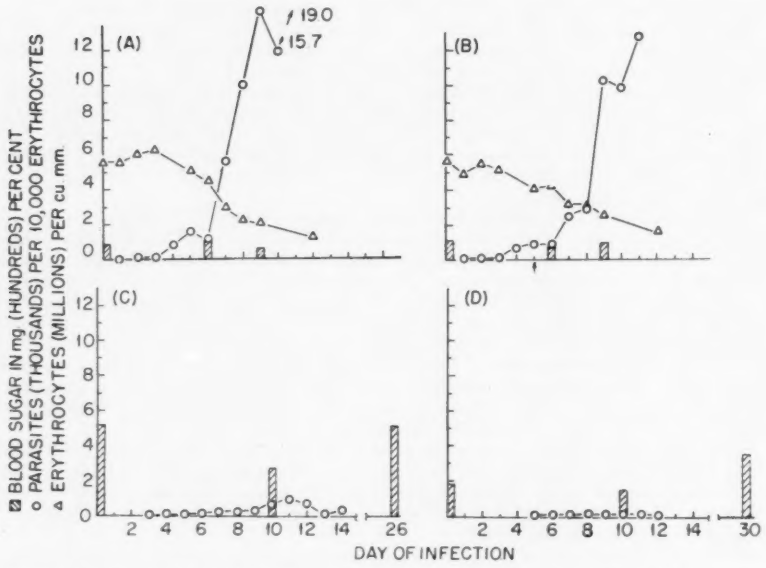

FIGURE 1. The levels of blood glucose, parasitemia, and (A and B) erythrocyte counts on diabetic and non-diabetic animals. IA is a control rat; B, a rat which received alloxan (time of administration indicated by arrow) but which did not become diabetic. C and D are representative rats which received alloxan and became diabetic.

markedly affected by the type of infection. When the infection was of a fulminating type with no crisis there was a rapid, consistent loss of erythrocytes. If, on the other hand, there was a mild infection in which the number of parasites never exceeded 1200 to 1300 per 10,000 erythrocytes, there was a gradual drop in erythrocytes followed by a rapid restoration to preinfection levels. In 1 rat in which the infection was characterized by a crisis and recovery, the blood cell count dropped to 1.620 million, but following the crisis it returned to normal levels.

The blood of 3 of the 10 rats was tested for glucose concentration before and during infection. In all these animals the glucose was reduced (fig. 1A). Inasmuch as our findings were in agreement with those of Mercado (1952), no attempts were made to determine the level of glucose for all control animals.

The course of infection of P. berghei *in rats treated with alloxan without becoming diabetic.* This group included 15 infected animals, weighing 116 to 288 g, which were treated with alloxan but did not become diabetic. With the exception of 1 animal which received 100 mg alloxan monohydrate per kilogram of body weight, all animals received 120 mg per kg of body weight. The levels of blood sugar before alloxan was administered averaged 105 mg per 100 ml (table I). The day following alloxan treatment, the average blood sugar was 104 mg per 100 ml, indicating that the dose of alloxan was insufficient to produce diabetes in these animals.

The course of *P. berghei* infections in this group was quite similar to that seen in the untreated controls (fig. 1B). At the beginning of the experiments, the mean erythrocyte count was 6.308 ± 0.153 million cells per cubic mm. When the infections had reached their greatest heights, the mean erythrocyte count was reduced to

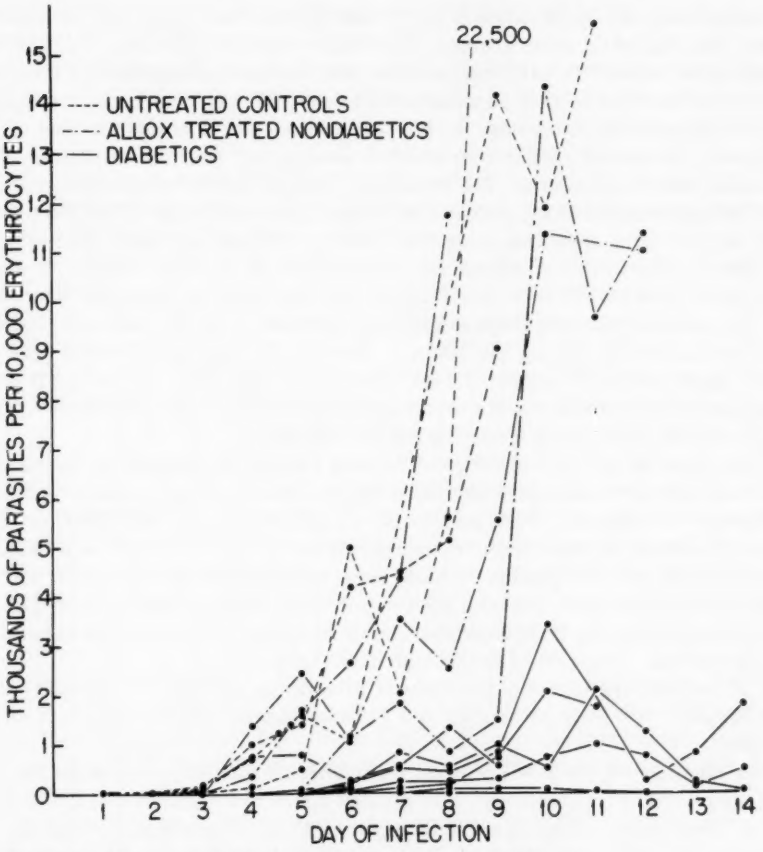

FIGURE 2. The course of infection in diabetic and non-diabetic rats.

2.291 ± 0.281 million cells per cubic mm. Average parasite counts at the height of infection were 9,334 parasites per 10,000 erythrocytes, ranging from 776 to 15,000. Statistical comparison (Pitman, 1937) of these counts to those of the untreated controls showed the level of significance to be $.50 > p < .60$.

Two animals of this group showed a considerable degree of innate immunity. Rat 9, which weighed 217 g, had only 776 parasites per 10,000 erythrocytes at the height of infection. Similarly, in another rat, which weighed 264 g, only 990 parasites per 10,000 erythrocytes were seen at peak parasitemia. Both animals recovered from the infection.

In general, the course of infection in these animals was the same as that seen in rats not receiving alloxan (fig. 1A).

The course of infection of P. berghei *in diabetic rats.* The 9 animals of this group became diabetic after treatment with alloxan monohydrate (120 mg per kg of body weight) (table I). Since erythrocyte counts during the course of *P. berghei* infections had been determined in many animals and found to be very similar, counts were made on only 2 animals in this group. At the peak of parasitemia, the average

parasite count was 2,580 parasites per 10,000 erythrocytes which was significantly lower than that of the other groups. Statistical comparison (Pitman, 1937) of this group to the untreated controls and to those which received alloxan without becoming diabetic showed the level of significance to be $p = .001$. In only 1 rat was there a level of parasitemia comparable to that seen in the other 2 groups; it died of the infection. It received alloxan after infection was patent. Another rat probably died of causes other than malaria. The remaining 7 animals recovered from the infection.

The percentage of blood glucose after alloxan treatment ranged from 180 to over 500 mg, averaging somewhat more than 340 mg. During infection, blood glucose dropped in all animals. In certain rats which recovered from the infection, the percentage of blood sugar rose to levels higher than that observed during the infection.

The initial erythrocyte count in rat 1 was 5,930,000 cells per cubic mm, and initial blood sugar was 107 mg per 100 ml. After alloxan was administered (day 5), blood sugar rose to 270 mg per 100 ml. Peak parasitemia (day 8) was 1,331 parasites per 10,000 erythrocytes; the erythrocyte count at this point was 4,380,000. The level of blood sugar on day 9 was 168 mg per 100 ml.

Rat 2 was the only one of the group to show a degree of parasitemia approaching the levels seen in the untreated animals or the ones which did not respond to alloxan treatment with diabetes. Peak parasitemia was 12,090 parasites per 10,000 erythrocytes. Erythrocyte count at the beginning of infection was 6,010,000; at peak parasitemia, 1,790,000. Originally, the blood sugar concentration was 92 mg per 100 ml. After alloxan was given (day 5), the concentration of blood sugar rose to 215 mg per 100 ml, but by day 9 (peak parasitemia) it had fallen to the very low value of 42 mg per 100 ml. The rat died on the ninth day of infection.

In the following rats only the levels of parasitemia and blood sugar levels were determined. All received alloxan and became diabetic before infection with *P. berghei*.

In rats 3 and 4, the levels of blood sugar after alloxan was given were 334 and 315 mg per 100 ml, respectively. The greatest number of parasites was 2,070 in rat 3; the blood sugar had decreased to 82 mg per 100 ml. The animal died on day 12. In rat 4, the peak parasitemia was 3,460 and blood sugar concentration was 97 mg per 100 ml. The blood sugar level increased to 125 milligrams per 100 ml on day 30.

Rat 5 weighed 275 g. Its low level of parasitemia, 2,100 at peak, might be credited to natural resistance. The blood sugar level dropped from 280 mg per 100 ml on the day after alloxan to 119 mg per 100 ml on day 10 of the infection. After the rat recovered from the infection, the blood sugar was 128 mg per 100 ml.

Rats 6, 7, 8, and 9 showed blood sugar levels ranging from 180 mg per 100 ml to over 500 mg per 100 ml following the administration of alloxan. In all 4 animals, the blood sugar dropped during the course of infection, but rose again after they had recovered from the infection. Rat 5 (fig. 1D) showed a higher level after recovery (363 mg per 100 ml) than prior to infection (180 mg per 100 ml).

Figure 2 shows a comparison of the course of infection as shown by the number of parasites per 10,000 erythrocytes in representative rats from all groups: the untreated controls, the alloxan treated rats which did not become diabetic, and rats which became diabetic after alloxan treatment.

DISCUSSION

In general, the course of *P. berghei* infections in untreated albino rats was found

to be the same as that found by other workers (Fabiani et al, 1952; Galliard and Lapierre, 1951, and Mercado and Coatney, 1951).

It was also observed that blood sugar levels decreased during *P. berghei* infections in all 3 groups, as reported by Mercado (1952). Explanations advanced for the diminution are (1) the parasites use the blood sugar for their own metabolism (Fulton, 1939, and Mercado, 1952), (2) the presence of the malaria infection causes a general malaise in which the animals' food intake is decreased (Mercado, 1952), and (3) liver damage resulting from infection causes an upset in carbohydrate metabolism (Mercado and von Brand, 1954).

No significant difference in the course of infection was noted between the rats in the untreated group and those receiving alloxan but not rendered diabetic. Since the alloxan doses were ineffective in producing diabetes, there was presumably no physiological difference in the hosts; therefore, no difference in the infection would be expected. In addition, the fact that there was no difference in the infections in these 2 groups indicates that the alloxan itself had no toxic effect on the parasites.

The course of infection in diabetic rats was significantly different from the other 2 groups. Instead of an increased parasitemia as reported by MacDougall (1927) in hyperglycemic canaries infected with *P. cathemerium*, we observed a marked decrease in parasitemia. This could be attributed to differences in the avian and mammalian hosts, a difference in the parasites, or a combination of these factors.

Since glucose or, more particularly, its breakdown products serve as the source of energy for the metabolism of the malaria parasites, the disturbance of glucose metabolism in the absence of insulin could account for the decrease in parasitemia. Gomori and Goldner (1943) reported that animals rendered alloxan-diabetic showed ketonuria after 3 days when blood sugar levels were higher than 300 mg per 100 ml. Janes and Myers (1946) stated that rats which have severe diabetes are always on the borderline of ketosis. In view of these findings it would be interesting to investigate the effect of ketosis and acidosis produced by other treatment on the growth and development of malaria parasites in vivo.

The recent findings of Cooperstein and Lazarow (1958) that alloxan inactivates coenzyme A in pigeons is interesting in the light of the dependence of certain malarias on this enzyme for growth and reproduction (Trager, 1952).

The change from a hyperglycemic condition to levels approaching that of normalcy or below might be a result of several factors. It might well be that levels of glucose in the blood of rats made diabetic experimentally vary from day to day. A second possibility is that the presence of a large number of parasites might bring about an increased utilization of the excess glucose with concomitant decrease in sugar levels. In view of the return to a hyperglycemic condition following recovery of the infected individuals, this latter surmise is more attractive. Still further, some other physiological changes such as liver damage induced by infection might have played a role in the temporary regression of hyperglycemia.

It may be concluded that under conditions of our experiments, the usual course of infection of *P. berghei* in albino rats was modified as a result of the diabetic syndrome. Whether this same effect would be produced in other malaria infections in mammals, including those in man, is yet to be determined.

SUMMARY

The effect of alloxan diabetes on *P. berghei* infections in albino rats was found

to be an inhibitory one. The degree of parasitemia was significantly lower in diabetic animals than in untreated animals or alloxan-treated animals which did not become diabetic. This is in contrast to MacDougall's findings that hyperglycemia caused increased parasitemia in canaries infected with *P. cathemerium*.

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THE PREVALENCE OF CANINE TOXOPLASMOSIS IN MEMPHIS, TENNESSEE

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First recognition of the dog as a host for *Toxoplasma* was made by Mello (1910), who described an acute canine infection at Turin, Italy. Since that time there have been numerous reports of isolated clinical cases, and canine toxoplasmosis now is known from all the continents and most of the inhabited regions of the world except the polar areas. The pertinent literature was reviewed by Habegger (1953) and by Lainson (1956). Cole, Prior et al (1953) reported epizootics in which 115 animals from 2 kennels died as a result of infection with this parasite.

A few serological surveys have been carried out to determine the frequency of *Toxoplasma* antibodies in dogs from different parts of the world. These studies, which were reviewed by Lainson (1956), suggest that antibodies to *T. gondii* are widely prevalent in the dog population, thereby indicating that this animal becomes infected with considerable frequency and hence possibly may be an important source for human infection. As part of a broad epidemiological approach to the problem of transmission of human toxoplasmosis in the area around Memphis, Tennessee, we undertook the present 2-year study to determine how frequently we could detect antibodies and isolate parasites from the dogs of this area.

MATERIALS AND METHODS

All the dogs in our study were unlicensed stray animals or rejected pets from the Memphis pound. No other selection was made. Nearly all of the animals were in apparent good health when examined. During 1954, a total of 134 dogs was used. Blood serum from each was tested for *Toxoplasma* antibodies by the Sabin-Feldman dye test, using the original procedure stipulated by Sabin and Feldman (1948) and redescribed by Feldman (1954). This included inactivation of the test sera at 56 C for 30 minutes.

In addition, portions of brain, liver and spleen were taken from each dog, triturated together in physiological saline, and inoculated intraperitoneally into white mice in an attempt to isolate *Toxoplasma* organisms. Two mice were used for the original passage in each series, and 2 additional blind passages in mice were made according to the scheme shown in figure 1, which is a modification of the method used by Jacobs et al (1952) to isolate *Toxoplasma* from wild pigeons. One week after the initial inoculation, a first passage mouse was sacrificed and its entire brain, plus portions of liver and spleen, was triturated in saline and used to start the second or "B" passage. One week later, one of the "B" mice was similarly sacrificed to start the "C" passage. The remaining "A" and "B" mice were sacrificed 4 weeks after inoculation and their tissues were inoculated into the "C" passage mice to reinforce any organisms which might have been transferred in the earlier passages. It was hoped that this technique would allow us to detect either rapidly proliferating organisms or those which might develop more slowly. "C" passage mice were sacrificed at the end of 6 weeks. Smears of peritoneal fluid, liver, and brain were made from each mouse when it died or was sacrificed at the end of the designated periods.

Because Erichsen and Harboe (1953) had found that cortisone increases the susceptibility of mice to strains of *Toxoplasma* which are not highly virulent, we gave each of our mice 5 mg of

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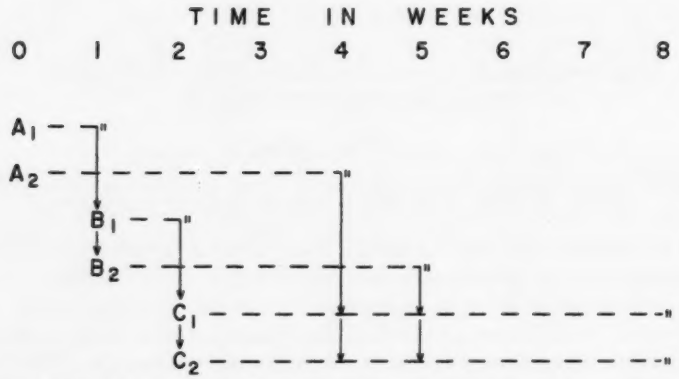

FIGURE 1. The scheme of animal passages used in attempts to isolate *Toxoplasma* by inoculation of tissues from suspected hosts into laboratory mice. (See text).

cortisone acetate intramuscularly at the time of inoculation. Penicillin and streptomycin were added to the inoculum to control bacterial contamination.

Because no *Toxoplasma* infections were detected by mouse inoculations in the 1st series of 134 dogs, a 2d series was studied using the dye test as a screening procedure. By this method only animals having titers of 1:16 or higher were selected for further testing by inoculation of tissues into mice. This eliminated the considerable effort involved in making mouse inoculations from dogs with low-titer or negative dye tests, since the probability of recovering *Toxoplasma* seemed remote in these cases.

During 1955 a total of 663 dogs thus was screened by the dye test. Mouse inoculations, using the techniques described previously, were made from 54 of the dogs having titers of 1:16 or higher. In most cases liver and spleen were not employed so that larger amounts of brain tissue could be used for the inoculum.

RESULTS

Results of the serological and parasitological studies on both series of dogs are summarized in table I. Of 137 dogs tested for *Toxoplasma* antibodies in the 1st

TABLE I. *Summary of dye test results and attempts to recover* Toxoplasma *by animal inoculations. (Data indicate the number of dogs at each antibody level.)*

	1st (unscreened) series		2nd (screened) series	
	Dye test	Animal inoc.	Dye test	Animal inoc.
Total tested	137	137	663	54
D.T. Pos. all titers	62 (45.3 pct)	62	Not tested	
" Pos. < 1:16	41 (29.9 pct)	41	Not tested	
" Pos. ≥ 1:16	21 (15.3 pct)	21	106 (16.0 pct)	54
" Neg.	75 (54.8 pct)	75	}	
" Pos. undil.	33 (24.1 pct)	33	} 557 (84.0 pct)	Not tested
" " 1:4	8 (5.8 pct)	8	}	
" " 1:16	11 (8.0 pct)	11	52 (7.8 pct)	26
" " 1:64	6 (4.4 pct)	6	41 (6.2 pct)	20
" " 1:256	4 (2.9 pct)	4	10 (1.5 pct)	5*
" " 1:1024	0	0	3 (0.5 pct)	3*

* *Toxoplasma* recovered from 1 dog in each of these 2 groups.

(unscreened) series, 62 (45.3 percent) were positive when all titers were considered, while 41 (29.9 percent) were positive at the undiluted or 1:4 level and 21 (15.3 percent) were positive at titers of 1:16 or higher. The positive sera showed a bimodal distribution with a large number of undiluted positives, as we have described in human populations (Gibson et al, 1956), and a smaller modal peak at the 1:16 titer. Tissues from all dogs in this series were inoculated into mice, but no *Toxoplasma* infections were detected.

In the 2d (screened) series, 663 dogs were tested for antibodies by the dye test. Of this number, 106 dogs (16.0 percent) had antibodies at a titer of 1 : 16 or higher. Lower titers were not determined in this series. Of the 106 dogs showing titers of 1 : 16 or above, 54 were used for parasite isolation studies by inoculation of tissues into laboratory animals. Two *Toxoplasma* infections were thus detected. Both infected dogs had high dye test titers, 1 : 256 and 1 : 1,024 respectively. One dog was a female, the other a male; both were adults. In both cases the parasites were found in tissue smears from 1st and 2d passage mice only after all mice from the series had been sacrificed, and we thus could not recover the strains. The parasites did not adapt well to mice, since in both instances the organisms were fairly abundant in mice of the first passage but rare in the second passage, while none was found in 3d passage mice.

Table II shows the relation of age and sex to the prevalence of antibodies at

TABLE II. *Dye test results by age and sex.*

Age	Sex	All Titers* (1)		Titers $< 1:16$* (2)		Titers $\geq 1:16$** (3)	
		Number tested	Percent positive	Number tested	Percent positive	Number tested	Percent positive
Adult	Male	48	50.0	48	29.5	288	19.8
	Female	62	40.3	62	24.2	240	20.0
	Both	110	44.6	110	30.9	528	19.9
Juvenile	Male	14	57.2	14	21.5	100	9.0
	Female	13	38.5	13	30.8	172	7.6
	Both	27	48.2	27	25.9	272	8.1
All ages and both sexes		137	45.3	137	29.9	800	15.9

* Based on 137 dogs of the 1st (unscreened) series.
** " " 800 " " " " " " " and the 2d (screened) series combined.

various titers. Since determination of negative and low-titer sera was made only in the 1st series of 137 dogs, data from this group alone were used in calculating the effect of sex and age upon the prevalence of all titers combined (col. 1) and of titers less than 1 : 16 (col. 2). There was little difference in the prevalence of antibodies between males and females, either adults or juveniles, with respect to the low titers or to all titers combined.

Since all dogs in both series were tested at dilutions of 1 : 16 or higher, the prevalence of these higher titers as given in table II (col. 3) was calculated from all 800 animals. Here again there was little difference between males and females. Thus we failed to detect a difference in prevalence of antibodies between the sexes, either at high or at low titers. Statistical evaluation of the various data confirms this conclusion.

Comparison of the adults with the juveniles, however, reveals a considerably different situation. At low titers the difference in prevalence of antibodies between the 2 age groups is not striking, but at titers of 1 : 16 or above the adults show a decidedly higher proportion of positive sera (19.9 percent) than do the juveniles (8.1 percent). Statistical analysis gives a critical ratio of 4.3 for this difference, with a probability of less than 0.00006 that the observed difference is due to chance. Thus the difference in prevalence of antibodies between adult and juvenile dogs is highly significant. Admittedly, our criteria for distinguishing adult dogs from juveniles were difficult to define, being based chiefly on size in relation to breed, and the con-

dition and development of the genitalia. Even with this reservation the difference in prevalence rates is striking.

Table III presents a different analysis of the serological data to permit a com-

TABLE III. *Comparative prevalence of* Toxoplasma *antibodies in dogs from rural and urban environments.*

Environment	No. tested	Titers ≤ 1 : 4		Titers ≤ 1 : 16	
		No.	Percent	No.	Percent
Urban	82	19	23.2	14	17.1
Rural	42	19	45.2	4	9.5
			C.R. = 2.5		C.R. = 1.1
Total	124		P = 0.01		P = 0.27

parison of dogs from within the City of Memphis with those collected in the surrounding rural and suburban areas of Shelby County. Since city and county dogs normally were held in common pens at the city pound before being released to us, it was difficult to work out a system of recognition. Various dyes were tried in an effort to mark the rural dogs at the time of their capture, but these efforts were only partially successful due to practical difficulties involved in application of the dye, recognition of marked dogs when delivered to us several days after capture, and transfer of dye from one dog to another in the pens. For this reason only 124 animals are included in the data given in table III, representing dogs which we could definitely trace to either city or county collections. Low titers (less than 1 : 16) were much more prevalent among the rural dogs (45.2 percent) than among the urban dogs (23.2 percent). A difference of this magnitude would be observed by chance only once in a hundred times. For titers of 1 : 16 or higher, the prevalence rates (17.1 percent for urban dogs and 9.5 percent for rural dogs) suggest the opposite situation, but statistical treatment of the data indicates that a difference this great could occur 27 times in a hundred by chance alone. Hence the present data do not permit a conclusion as to whether there actually is a difference between urban and rural dogs with respect to the higher titers.

DISCUSSION

To our knowledge, the present report represents the first attempt to correlate, in the same series of dogs, the prevalence of *Toxoplasma* antibodies with the presence of spontaneous latent *Toxoplasma* infections as revealed by animal inoculation studies. It was feasible to carry out this type of study on a large series of animals only by adoption of the dye test as a screening procedure in our 2d series of dogs, thereby selecting for animal inoculation only those dogs having titers of 1 : 16 or higher. The validity of this screening procedure has been established by recent investigations of this laboratory (Gibson and Eyles, 1957; Jones et al, 1957).

In those studies, latent *Toxoplasma* infections in feral cats were detected in nearly all animals with dye test titers of 1 : 256 or higher and in some animals with titers of 1 : 16 or 1 : 64, but almost never in animals with titers below 1 : 16. The data given in table I suggest that the same relationship holds in canine toxoplasmosis, since parasites were found in 2 of the 8 dogs having titers of 1 : 256 or above, while the 46 dogs with lower titers failed to produce infections in the inoculated mice.

It is interesting to compare this low rate of recovery of parasites in dogs with the comparable situation in cats. Members of this laboratory completed a study in

which latent *Toxoplasma* infections were detected by animal inoculation studies in 21.6 percent of a large series of cats obtained from the same source as the dogs in the present investigation (Jones et al, 1957). These discrepant results suggest either that *Toxoplasma* infection is much less common in dogs than in cats or that the parasites are more difficult to detect in dogs. Referring to their failure to recover parasites from several experimentally infected dogs, Jacobs et al (1955) state "it appears, therefore, that the isolation of *Toxoplasma* from the tissues of dogs is fortuitous, and that it may not be accomplished even in animals with latent infections."

In reviewing the cases of spontaneous canine toxoplasmosis which had been reported in the literature, Fankhauser (1951) observed that only female dogs had been found infected. More recent studies have indicated, however, that males are not immune. One of the 2 infections reported in the present study was in a male dog. Furthermore, no significant difference between the sexes was observed in our studies with regard to the prevalence of antibodies or the distribution of titers, thus confirming similar observations of Otten et al (1951) and Lainson (1956).

Both Beattie (1955) and Jacobs et al (1955) have pointed out that young dogs are more susceptible to experimental infection with *Toxoplasma* than are older dogs. Lainson (1956) suggests that, this being so, the likelihood of detecting *Toxoplasma* antibodies among the lower age group is considerably diminished, because young infected animals usually die, and thus are not available for serological testing. Lainson postulates that this might account for his findings, in which 45.0 percent of the adult dogs which he tested in London had complement-fixing antibodies for *Toxoplasma*, while only 33.3 percent of the juvenile dogs gave positive results for the test. While the difference between these 2 groups is not significant statistically, it should be noted that Lainson performed his tests only at the 1:8 serum dilution. If he had also tested the sera at higher dilutions, he might have found a situation comparable to that in the present study, in which dye test antibodies were equally prevalent in young and old dogs at titers below 1:16, but showed a significantly greater prevalence in adult dogs at dilutions of 1:16 or above.

Although a number of investigators have studied the prevalence of canine toxoplasmosis in various parts of the world, it is difficult to make meaningful comparisons because different criteria have been used. Lainson (1956) has reviewed most of the pertinent reports. While specific comparisons cannot easily be made, these various studies leave no doubt that a high proportion of dogs throughout the world have chronic and inapparent infections, although the prevalence of such latent infections varies greatly from one locality to another.

The situation in the United States was studied by Cole, Docton et al (1953). Using the skin test, they found 76 positive reactions among 1,944 dogs tested at the Ohio State University Veterinary Clinic (3.9 percent), but dye tests failed to reveal antibodies in the sera of 387 healthy dogs or in the sera of 125 dogs representing numerous canine diseases other than toxoplasmosis. Additional studies were made in this country by Feldman and Miller (1956) using the dye test. At titers of 1:16 or higher they found antibodies as follows: Syracuse, N. Y., 28 percent (14 positives among 51 dogs tested); north-central Pennsylvania, 59 percent (30 positives among 51 tested); Fort Defiance, Arizona, 30 percent (7 of 23 dogs belonging to Navajo Indians).

The prevalence data in table II of the present report suggest that *Toxoplasma* antibodies occur with much less frequency in the Memphis area than in the various localities of the United States which were sampled by Feldman and Miller (1956). On the assumption that the dogs tested were indeed representative of the localities studied, it is evident that there is considerable variation in the prevalence of canine toxoplasmosis from one place to another. This is clearly illustrated by the finding of Feldman and Miller that antibodies occur with approximately the same frequency in the City of Syracuse and in the sparsely inhabited plateau country of Arizona, under extremely different environmental conditions, while they are nearly twice as prevalent in north-central Pennsylvania, where still other ecological factors prevail.

Although it is impossible at present to detect any pattern which satisfactorily explains the variable prevalence of canine toxoplasmosis, it is patently evident that latent infections by *Toxoplasma* are frequent and widespread in dogs. To what extent, if any, the dog serves as a reservoir for human infection is likewise obscure. It may well be true, as Jacobs et al (1955) have suggested, that because of their close association dogs and man may acquire the infection from the same source.

SUMMARY

Two series of dogs from the pound at Memphis, Tennessee, were studied by the Sabin-Feldman dye test to determine the prevalence of antibodies to *Toxoplasma gondii,* and by inoculation of tissues into laboratory mice to determine the frequency with which parasites could be isolated.

In the first series, 137 dogs were tested for serum antibodies and portions of liver, spleen and brain were inoculated into mice. Antibodies (all titers) were detected in 45.3 percent of the sera, with 29.9 percent having titers less than 1 : 16 and 15.3 percent showing titers of 1 : 16 or higher. No parasites were isolated from dogs of this series.

In the second series, 663 dogs were screened by the dye test at titers of 1 : 16 or higher. Antibody at these levels was found in 106 (16.0 percent) of the dogs, of which 54 were further tested by inoculation of brain tissue into mice. *Toxoplasma* infections were thus detected in 2 adult dogs, 1 female and 1 male, with antibody titers of 1 : 256 and 1 : 1,024 respectively.

Analysis of the serological findings on the 800 dogs of both series showed the following: (1) Antibodies were equally frequent in males and females. (2) Antibodies at low titers ($< 1 : 16$) were significantly more prevalent in rural dogs than in urban dogs, but at higher titers (1 : 16 or above) there was no significant difference. (3) Antibodies were equally frequent in juveniles and adults at the lower titers, but were significantly more prevalent in adult dogs at titers of 1 : 16 or higher.

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RESEARCH NOTE

QUANTITATIVE STUDIES ON POPULATIONS OF BITING MIDGE LARVAE IN SATURATED SOIL FROM TWO TYPES OF MICHIGAN BOGS (DIPTERA: CERATOPOGONIDAE).

The isolation of midge larvae and other small invertebrates from the psamolittoral of lakes has long been accomplished by washing sand, filtering, and pouring or rewashing the residue into a pan for examination. Bidlingmayer (1957, Mosquito News **17:** 292–294) demonstrated that the larvae of the salt marsh midge, *Culicoides furens* (Poey), could be isolated from soils other than sand by placing a 2-inch layer of sand atop a soil sample and flooding with water. During an overnight period the midge larvae would migrate into the sand above. This sand then was treated as beach sand and the larvae readily isolated.

Bidlingmayer's discovery that midge larvae will leave their normal soil habitat to migrate into sand placed upon it is of considerable significance to those interested in ascertaining midge larval populations in soils, comparing populations qualitatively and quantitatively in the same or different soil habitats at one or at different times, and in associating the immature stages with their respective adult forms.

During the summer of 1959 Bidlingmayer's technique, with some modifications (Williams, 1960, Ecology. In press), was used to isolate midge larvae from a *Sphagnum* and a peat bog in Cheboygan County, Michigan, to obtain larvae for rearing purposes. In conjunction with this project a quantitative larval study was also made of 25 soil samples from each bog taken between June 22 and August 12. Each sample, from the top 2 inches of soil, occupied an area of 64 square inches, or a volume of 128 cubic inches.

In view of Bidlingmayer's findings that when a known number of midge larvae was placed in marsh soil and recovered the following day by the use of his technique, the average percent recovered in a series of replicates was 70, I permitted 6 soil samples from each of the 2 bogs to stand a second period of 20+hours under a topping of fresh sand. Larvae were then isolated from this sand and counted. It was assumed that, if the number of larvae isolated from the first toppings averaged 70 percent or more of the total number isolated from the 2 toppings in each group of 6 samples, close to 100 percent of the larvae would be recovered from soil samples which were permitted to stand for 40+hours under a single topping of sand.

Bryant's bog. The soil composition of the Bryant's Bog material studied was of thickly matted *Sphagnum*, *Sphagnum* roots, and decomposed organic matter. Twenty-five samples were studied from different locations around the edge of the bog mat but all taken from within 8 inches of the water at the center of the bog. From the 6 samples from which 2 sand toppings were studied over a period of 40+hours 76 percent (896) of all larvae recovered were taken from the first sand toppings and 24 percent (281) were recovered from the second sand toppings. The other 19 samples were permitted to stand for 40+ hours before examining the original sand topping. (From the 25 samples, averaging 1.38 cubic inches of soil, a total of 4,430 midge larvae was recovered.) Fourteen percent (620) of them were dead when recovered (they were alive when they migrated into the sand toppings; therefore, most of them probably were killed by the removal and washing of the sand).

Reese's bog. Reese's Bog is composed largely of a brown, rather coarse, and moderately decayed woody peat, known as Rifle peat, which ranges from 1 to 2 feet in thickness and overlays a yellowish-brown, wood-fibrous peat. The water table fluctuates but may be from surface level to 18 inches below. Where the water table is at the surface there are areas around the water which are free of any vegetation, others with moss but no leaf litter. The soil samples studied were taken from such areas adjacent to the water. From the 6 samples of which 2 sand toppings were studied, over a period of 40+hours, 80.3 percent (837) were recovered from the first sand toppings and 19.6 percent (304) from the second. A total of 4,012 midge larvae were recovered from 25 samples, averaging 1.22 per cubic inch, after each had remained at least 40 hours under a sand topping. Of this total number 16 percent (641) were dead when recovered.

It would appear that in Cheboygan County, Michigan, large numbers of midge larvae may be found in well-saturated bog soils irrespective of the physical nature of the soil. It is expected, however, that the qualitative nature of these populations may be different (to be reported on in a later paper). It is generally recognized that more midge larvae are found in the top inch of soil than in the second. Therefore, the average number of larvae in the top inch would presumably be somewhat higher than the average in the top 2 inches reported here. There was no significant difference in the average number of midge larvae recovered at weekly intervals during the more than 7 weeks of this study.

In comparative studies of quantitative populations from different soil types at least 40+ hours should be allowed for the migration of larvae into the sand toppings. If time does not permit this, then 20+hours may be used, but the difference in the percent of larvae migrating within this period from different types of soils should be ascertained and this difference considered in estimating total populations within soil samples from different soil habitats.

This is a contribution from the University of Michigan Biological Station. This study was made possible through moneys from grant 2E-55 from the National Institutes of Health and grant G-4978 of the National Science Foundation.—ROGER W. WILLIAMS, *School of Public Health and Administrative Medicine, Columbia University.*

THE USE OF CHAETOTAXY IN THE IDENTIFICATION OF LARVAL TICKS (ACARINA: IXODIDAE)*

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The specific determination of the larval stage of ixodid ticks has been a particularly troublesome problem for systematists for many years. As a consequence, relatively little has been learned about various aspects of the biology of this stage and the role of the larvae in disease transmission. Our meager knowledge of the systematics of larval ticks can be attributed to a number of factors; namely, the small number of known reliable characters available for inclusion in keys, the inadequate descriptions and illustrations of the relatively few species reared in the laboratory from identified females, and the virtual impossibility of associating field-caught larvae of most species with the corresponding adult.

The lack of reliable recognition characters has been the most serious deterrent to a better understanding of the systematics of larval ticks. The present study was undertaken for the purpose of seeking additional characters to overcome this handicap. It was found that chaetotaxy, long used successfully in separating certain groups of mites, offered a number of useful characters for making a key to genera of larval ticks of the world. A key was designed also which could separate all of the species of larval ticks presently available for study, but this key included such a small proportion of the total number of ixodid species that it has little, if any, practical value. Thus, a great need exists for the characterization of more species of larval ticks from all geographical regions. This characterization should be based upon laboratory reared larvae derived from positively identified females. The description of larvae by this method would lead to a better understanding of the tick fauna of each region and eventually to a monograph of the systematics of larval ticks on a world basis. Some progress in these respects has been made by several studies in the USSR and the completed, but as yet unpublished, study of the larval ticks of the eastern United States by the authors. The key to the species of *Ixodes* from the latter study has been included in this paper to demonstrate its practicality.

When it became apparent early in the course of this study that the setae of larval ticks were arranged in definite distributional patterns a system was devised for naming them according to their topographical location. Later it was discovered that two Russian acarologists, B. V. Lototsky (1949) and L. V. Glashinskaya-Babenko (1949), had investigated the chaetotaxy of larval ticks occurring in the USSR and also had proposed systems of nomenclature. Of these systems the one proposed by Glashinskaya-Babenko lends itself better for the practical determination of species, and it also coincides very closely with the findings reached independently in the present study. For these reasons the terminology of Glashinskaya-Babenko has been adopted, although some new names have been proposed for setae not named by this author.

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MATERIALS AND METHODS

The following 56 species from the 6 continents designated were examined by the authors and at least 25 specimens of each were examined except where noted by the number in parenthesis following the specific name: *North America, Amblyomma americanum* (Linné), 1758; *A. dissimile* Koch, 1844; *A. maculatum* Koch, 1844; *A. tuberculatum* Marx, 1894 (8); *Anocentor nitens* (Neumann), 1897; *Boophilus annulatus* (Say), 1821; *Dermacentor andersoni* Stiles, 1908; *D. albipictus* (Packard), 1869; *D. parumapertus* Neumann, 1901; *D. variabilis* (Say), 1821; *Haemaphysalis chordeilis* (Packard), 1869; *H. leporispalustris* (Packard), 1869; *Ixodes angustus* Neumann, 1899 (3); *I. brunneus* Koch, 1844 (15); *I. bishoppi* Smith and Gouck, 1947 (10); *I. dentatus* Marx, 1899; *I. cookei* Packard, 1869; *I. marxi* Banks, 1908; *I. muris* Bishopp and Smith, 1937; *I. scapularis* Say, 1821; *I. texanus* Banks, 1909; *South America, A. cajennense* (Fabricius), 1787; *Africa, A. gemma* Dönitz, 1909; *A. hebraeum* Koch, 1844; *A. variegatum* (Fabricius), 1794; *B. annulatus* (Say), 1821; *B. decoloratus* (Koch), 1844; *B. fallax* Minning, 1934; *H. erinacei* Pavesi, 1884; *H. leachii* (Audouin), 1827; *H. silaceae* Robinson, 1911; *Hyalomma dromedarii* Koch, 1844; *Hy. excavatum* Koch, 1844; *Hy. rufipes* Koch, 1844; *Hy. truncatum* Koch. 1844; *I. festai* Rondelli. 1926; *I. pilosus* Koch, 1844 (4); *I. rubicundus* Neumann, 1904; *Margaropus winthemi* Karsch, 1879; *Rhipicephalus appendiculatus* Neumann, 1901; *R. compositus* Neumann, 1897; *R. evertsi* Neumann, 1897; *R. humeralis* Rondelli, 1926; *R. oculatus* Neumann, 1901; *R. pulchellus* Gerstäcker, 1873; *R. pravus* Dönitz, 1910; *R. sanguineus* (Latreille), 1806; *R. simus* Koch, 1844; *R. tricuspis* Dönitz, 1906; *Rhipicentor nuttalli* Cooper and Robinson, 1908; *Australia, A. triguttatum* Koch, 1844; *B. microplus* (Canestrini), 1887; *H. bispinosa* Neumann, 1897; *I. kohlsi* Arthur, 1955 (9); *I. holocyclus* Neumann, 1899; *I. tasmani* Neumann, 1899 (8); *Europe, D. marginatus* (Sulzer), 1776; and *Asia, H. bispinosa* Neumann, 1897.

In addition to the above mentioned species information concerning the following 25 species from the USSR was obtained from published records wherein the descriptions and illustrations were considered adequate: *B. calcaratus* (Birula), 1895; *D. nuttalli* Olenev, 1928; *D. silvarum* Olenev, 1931; *H. concinna* Koch, 1844; *H. punctata* Canestrini and Fanzago, 1877; *H. warburtoni* Nuttall, 1912; *Hy. plumbeum* (Panzer), 1795; *I. apronophorus* Schulze, 1924; *I. berlesei* Birula, 1895; *I. crenulatus* Koch, 1841; *I. kazakstani* Olenev and Sorokoumov, 1934; *I. lagodechiensis* Dzarparidze, 1950; *I. laguri laguri* Olenev, 1929; *I. occultus* Pomerantzev, 1946; *I. persulcatus* Schulze, 1930; *I. plumbeus* Leach, 1815; *I. putus* (Pickard-Cambridge), 1876 = *I. uriae* White, 1852; *I. redikorzevi* Olenev, 1927; *I. ricinus* (Linné), 1758; *I. stromi* Filippova, 1957; *I. tauricus* Vschivkov and *Filippova*, 1957; *I. trianguliceps* Birula, 1895; *I. vespertilionis* Koch, 1844; *R. bursa* Canestrini and Fanzago, 1877–78; and *R. rossicus* Yakimov and Koh!-Yakimov, 1911.

The setae are seen best when the larvae are mounted on slides and studied by means of phase microscopy. Hoyer's medium was found to be the most satisfactory substance for preparing whole mounts of larval ticks. The procedure was as follows: Larvae were placed in a 5 ml beaker containing 2 ml of lactic acid which was then heated to a temperature just below boiling. For unengorged specimens 1 to 2 minutes after the first fumes of lactic acid were given off was sufficient for clearing, but a longer time was required for engorged larvae. When the lactic acid cooled the specimens were washed in water to avoid crystallization of excess acid. Following this, the larvae were mounted, and the slides were placed in an oven at 35 C for 24 hours to hasten solidification of the medium as well as to clear further the various cuticular structures.

All of the setae are named according to their topographical position, i.e., body setae, anal setae, setae of the basis capituli, palpal setae, coxal setae, and tarsal setae. The terminology used for the body setae is that of Glashinskaya-Babenko (1949) but that for all of the other regions is new. The setae on the segments of the legs, except for the coxae and tarsus I, have not been named because of their uniformity in all of the species examined.

In some cases variations were observed in the setal pattern, i.e., a seta of a pair might be lacking or might be duplicated, but in spite of this variation the normal pattern is nevertheless usually discernible.

The body setae consist of 4 groups dorsally (figs. 1, 3), 4 groups ventrally and a pair of anal setae (figs. 2, 4). The dorsal groups are the scutal (Sc), marginal dorsal (Md), central dorsal (Cd), and supplementary (S). The ventral groups are the sternal (St), preanal (Pa), premarginal (Pm), and marginal ventral (Mv); the anal setae (A) are on the anal plate.

The setae of the basis capituli occur only on the ventral surface and consist of 1 pair in all of the species examined except in the genus *Ixodes* where 2 pairs are always present (Ph_1 or Ph_1 and $_2$) (figs. 2, 4). Setae are present on palpal articles 2, 3, 4, but because the setae on article 4 are so numerous and so difficult to count they have not been named. Article 3 usually has 4 setae on the dorsal surface and 2 on the ventral surface (fig. 12). These are the dorsal terminal (Dt), dorsal subterminal (Dst 1 and 2), dorsal anterior lateral (Dal), ventral terminal

(Vt) and the ventral subterminal (Vst). In some species of *Ixodes* an extra dorsal anterior lateral seta (Dal 2) is present and this makes a total of 7 setae on this segment. Article 2 also has 4 setae on the dorsal surface and 2 on the ventral surafce (fig. 12). These are the dorsal median lateral (Dml), dorsal basal lateral (Dbl), dorsal median mesial (Dmm), dorsal submedian (Dsm), ventral median mesial (Vmm) and the ventral submedian (Vsm). Occasionally the division between articles 2 and 3 is not very apparent and this makes it difficult to ascertain whether a seta occurs on article 2 or 3. This is especially true of the dorsal median lateral seta which is found very near the region of separation of these 2 articles.

The coxal setae are named according to their location on the coxae (fig. 9). Those on coxa I are the median mesial (C_1mm), anterior lateral (C_1al) and posterior lateral (C_1pl); those on coxa II are the anterior lateral (C_2al) and posterior lateral (C_2pl); and those on coxa III are the anteriol lateral (C_3al) and posterior lateral (C_3pl). The only exception to the number of setae located on the coxae was observed in the genus *Ixodes* where *I. dentatus, I. scapularis* and *I. bishoppi* had an extra anterior lateral seta (C_3al_2) on coxa III.

The setae on tarsus I occur dorsally and ventrally; those on the dorsal surface form different groups which are named according to the position they occupy in respect to Haller's organ (fig. 11), but those on the ventral surface were the same (fig. 10) in all ixodid ticks. Excluded from consideration are the sensory setae located within the anterior depression and within the capsule of Haller's organ. The group anterior to Haller's organ is termed the prehalleral (Pha), that in the immediate vicinity of the organ is the halleral (Ha) and the group posterior to Haller's organ is the posthalleral (Poh). For convenience the dorsal tarsal setae can be represented by a formula indicating the arrangement of the individual setae. For example, the formula 1:1:2:2:2:2 represents the arrangement in *Dermacentor variabilis* wherein 2 prehalleral setae are in tandem, followed by a pair of halleral setae and 3 pairs of posthalleral setae (fig. 5).

RESULTS AND DISCUSSION

Chaetotaxic features found to have value in separating genera of larval ticks include the distribution of the setae on certain parts of the body, the positional relationship of the setae to certain body structures and to other setae, the number of setae on a particular structure of the tick and the nature of the setae. The present discussion is limited primarily to these various features of chaetotaxy, and only casual reference is made to other morphological features that have value as diagnostic characters since these features are relatively well known. Included in this latter group are such features as the presence or absence of the anal groove, the shape of the palpi, the number of palpal segments, the number of spurs on the coxae, the presence or absence of festoons, the number of festoons, the presence or absence of eyes, and the shape of the basis capituli.

An analysis and comparison of the 3 main groups of setae, the body setae, the capitular setae and those of the legs, for 10 genera involving 81 species show that all of the genera, except *Ixodes,* are very similar (table I). This distinct isolation of the larvae of the genus *Ixodes* from those of all the other genera lends support to the long established division of the family Ixodidae on the basis of adults into 2 sections, the Prostriata and the Metastriata, with the genus *Ixodes* in the former and the remainder of the ixodid ticks in the latter. However, no features of chaetotaxy investigated in this study appeared to lend support to the division of the family Ixodidae into the 3 subfamilies, Ixodinae Vitzthum, 1941, Amblyomminae Neumann, 1911, and Rhipicephalinae Vitzthum, 1941.

Although the distribution of the setae when viewed in this manner easily separates the family Ixodidae into 2 sections the uniformity of setal distribution of these 3 groups in the Metastriata does not permit any further separation. However, certain individual groups of setae namely, the marginal dorsal, the marginal ventral and the tarsal, show differences which can be utilized for this purpose. The marginal dorsal setae of the body group have the greatest value for generic separation, and the

TABLE I. *Comparison of the chaetotaxy of several genera of larval ticks based on 81 species.**

Genera	No. of species	Body (Pairs)										Legs
		Ant. to dorsal Ss	Sc	Md	Cd	S	St	Pa	Pm	Mv	A	Tarsus I (dorsal)
*Ixodes***	31	—	3–5	6–10	2–6	0–8	3	1–3	3–7	3–5	1	4 : 4 : 2
Amblyomma	9	2	3	8	2	0	3	2	4	5	1	2 : 2 : 2 : 2 : 2
Haemaphysalis	9	2	3	8	2	0	3	2	4	4	1	2 : 2 : 3 : 1 : 2
Dermacentor	7	3	3	8	2	0	3	2	4	5	1	1 : 1 : 2 : 2 : 2 : 2
Anocentor	1	3	3	8	2	0	3	2	4	5	1	1 : 1 : 2 : 2 : 2 : 2
Rhipicephalus	12	4	3	8	2	0	3	2	4	5	1	1 : 1 : 2 : 2 : 2 : 2
Rhipicentor	1	3	3	8	2	0	3	2	4	5	1	1 : 1 : 2 : 2 : 2 : 2
Hyalomma	5	4	3	8	2	0	3	2	4	5	1	1 : 1 : 2 : 2 : 2 : 2
Boophilus	5	5	3	8	2	0	3	2	4	4–5	1	1 : 1 : 2 : 2 : 2 : 2
Margaropus	1	5	3	8	2	0	3	2	4	5	1	1 : 1 : 2 : 2 : 2 : 2

Ss, Sensilla sagittiformia; Sc, Scutal; Md, Marginal dorsal; Cd, Central dorsal; S, Supplementary; St, Sternal; Pa, Preanal; Pm, Premarginal; Mv, Marginal ventral; A, Anal.
* Includes information from the literature. ** *Ixodes kohlsi* not included. See table II.

marginal ventral setae of the body group and the dorsal setae of tarsus I of the leg have somewhat lesser value. The value of the marginal dorsal setae was not in the total number present but rather in their relationship to the sensilla sagittiformia. These are small oval sensory structures in the integument, and 8 of these are present in all larval ixodid ticks except the genus *Ixodes* where they are absent. The 2 located dorsally in the posterior lateral margin of the body have an important positional relationship to the marginal dorsal setae, whereas, the others which are located behind each of the 6 coxae have no significance in this system of diagnosis (figs. 1, 2).

All of the genera in the section Metastriata have 8 marginal dorsal setae, but the number of these setae located anteriorly to the dorsal sensillum sagittiforme on each side varies. *Amblyomma* and *Haemaphysalis* have 2 marginal dorsal setae anterior to the dorsal sensillum sagittiforme; *Dermacentor, Anocentor* and *Rhipicentor* have 3 present in this position, *Rhipicephalus* and *Hyalomma* have 4 and *Boophilus* and *Margaropus* have 5. These findings are supported also by the illustrations of larvae of *Rhipicephalus, Hyalomma* and *Boophilus* prepared by Dinnik and Zumpt (1949) and of larvae of *Haemaphysalis, Dermacentor, Rhipicephalus, Hyalomma* and *Boophilus* prepared by Serdyukova (1955).

Glashinskaya-Babenko (1949) also reported another use for the marginal dorsal setae. She noted that the more medial position of marginal dorsal 3 in relation to the body margin in the genus *Dermacentor* would separate this genus from *Hyalomma* and *Rhipicephalus* where this seta was located closer to the margin of the body. Although this feature was found to work satisfactorily with the material available in the present study, the relationship of the marginal dorsal setae to the sensillum sagittiforme was easier to use and was not variable.

The marginal ventral setae which numbered 5 in all of the Metastriata except in the genus *Haemaphysalis* where 4 were present might be used for diagnostic purposes. Glashinskaya-Babenko (1949), however, reported the occurrence of 4 to 5 pairs of marginal ventral setae in *Boophilus calcaratus*. This was not observed in 4 species of *Boophilus* examined in the present study where all had 5 pairs and, although this possibly reduces the value of this feature for generic separation it might find application for specific diagnosis. Also, Glashinskaya-Babenko has utilized the more posterior position of marginal ventral setae 1 in relation to the premarginal setae 2 in *Rhipicephalus* to separate this genus from *Hyalomma* where these 2 pairs of setae are approximately at the same level. The value of this character was con-

firmed in the present study, but its use was found to be limited because of its variation in some species.

Although the usefulness of setal length as a diagnostic character was not determined in the present study, Glashinskaya-Babenko (1949) used this feature to separate *Haemaphysalis* and *Boophilus*. It was found that premarginal setae 1 and 2 and premarginal setae 3 and 4 were of equal length in *Haemaphysalis* but that premarginal setae 1 and 2 were markedly longer than premarginal setae 3 and 4 in *Boophilus*.

The last group of setae which might be used for diagnostic characters are those found on the dorsal surface of tarsus I, and their arrangement on the tarsus can be expressed by means of formulae (figs. 5, 6, 7, 8). The alignment of the setae in the genus *Ixodes* was 4:4:2, in *Amblyomma* 2:2:2:2:2 and in *Haemaphysalis* 2:2:3:1:2. The remaining 7 genera in the section Metastriata all showed an arrangement expressed as 1:1:2:2:2:2. Care must be taken in the use of this character because the possibility exists of confusing *Hyalomma* and *Amblyomma*. In this case the anterior pair of prehalleral setae of *Hyalomma* are very close together and might be interpreted mistakenly as being opposite each other rather than in tandem. On the other hand, the dorsal tarsal setae can be of great value in certain instances: for example, the larvae of *Dermacentor variabilis* and *Amblyomma maculatum*, which occur in the southeastern United States, are easily confused because of the great similarity of the basis capituli and coxal spurs in these two species. However, the alignment of the setae on tarsus I readily separates them according to genus and makes specific determination easier.

The following key to the genera of larval ticks of the world utilizes some of the features that have been discussed above. It includes all of the long established genera except *Aponomma* for which no laboratory reared material was available. It is anticipated that no difficulty will arise in incorporating this genus into the key when such material is studied eventually.

At the present time no differences could be found that would separate *Anocentor* and *Margaropus* from *Dermacentor* and *Boophilus*, respectively. This study tends to confirm the opinion of some tick systematists who have long remained unconvinced of the validity of *Anocentor* and *Margaropus*.

KEY TO THE GENERA OF LARVAL TICKS

1. Sensilla sagittiformia absent. With 2 pairs of posthypostomal setae. Anal groove present. *Ixodes*
   Sensilla sagittiformia present. With 1 pair of posthypostomal setae. Anal groove absent. .. 2
2. Palpi long, with 4 articles (article 1 present). 3
   Palpi short, with 3 articles (article 1 absent). 5
3. With 2 marginal dorsal setae anterior to the sensillum sagittiforme on each side. Body with 11 festoons. ..... *Amblyomma*
   With 3 or 4 marginal dorsal setae anterior to the sensillum sagittiforme on each side. Body with 7 or 9 festoons. 4
4. With eyes raised and protruding. With 4 marginal dorsal setae anterior to the sensillum sagittiforme on each side (occasionally 5). Body with 7 festoons. ..... *Hyalomma*
   With eyes flat and not protruding. With 3 marginal dorsal setae anterior to the sensillum sagittiforme on each side. Body with 9 festoons. ..... *Dermacentor* and *Anocentor*
5. With 2 or 3 marginal dorsal setae anterior to the sensillum sagittiforme on each side. ... 6
   With 4 or 5 marginal dorsal setae anterior to the sensillum sagittiforme on each side. ... 7
6. Without eyes. With 2 marginal dorsal setae anterior to sensillum sagittiforme on each side. Palpi with second article usually extended laterally. Body with 11 festoons. *Haemaphysalis*
   With eyes. With 3 marginal dorsal setae anterior to sensillum sagittiforme on each side. Palpi with second article not extended laterally. Body with 9 festoons. ..... *Rhipicentor*

7. With 5 marginal dorsal setae anterior to the sensillum sagittiforme on each side. Body without festoons. Tips of palpi blunt. Lateral sides of basis capituli rounded.
Boophilus and *Margaropus*

With 4 marginal dorsal setae anterior to the sensillum sagittiforme on each side. Body with 9 festoons. Tips of palpi pointed or blunt. Lateral sides of basis capituli rounded or acute.
Rhipicephalus

In general, the chaetotaxic and other morphological features useful for the separation of genera also have application in the separation of species of larval ticks. In addition, certain features which had no generic value were found to be useful at the specific level. Among these are the shape of the basis capituli and the hypostome, the number of rows of hypostomal teeth, as well as the number in each file, the nature of the auriculae when present, the shape and the size of the spurs on the coxae and the trochanters, and the shape and proportions of the scutum, the palps, and tarsus I. Chaetotaxy also provides a variety of features which appear to be useful for separating species of larval ticks, but their full value cannot be appraised in view of the relatively few described species for most of the genera. This is particularly true of those in the group Metastriata where the larvae of the least number of species are known and where the number and disposition of setae are uniform (table I). On the other hand, several reliable chaetotaxic features were found in the genus *Ixodes* of the group Prostriata where the larvae of more species of ticks have been described and where pronounced variations in chaetotaxy were observed. Among the more important of these are the number of setae on various parts of the body, setal length, the nature of the setae and their positional relationship to certain other setae.

Filippova (1958) has utilized some of these chaetotaxic features as well as other morphological features in a study of larval *Ixodes* occurring in the USSR. This author regards *Ixodes putus* (Pickard-Cambridge), 1876 = *I. uriae* White, 1852, as belonging to a separate genus, *Ceratixodes* Neumann, 1902, and subdivides *Ixodes* Latreille, 1795, into the subgenera *Ixodes s. str.*, *Eschatocephalus* Frauenfeld, 1853, *Ixodiopsis* Filippova, 1957, *Pholeoixodes* Schulze, 1942, *Scaphixodes* Schulze, 1941, and *Exopalpiger* Schulze, 1935. Most of these 6 subgenera had been established on the basis of adult characters. It is interesting to note that larval characters, including some chaetotaxic, also lend considerable support to these groupings. The 9 species of *Ixodes* examined in the present study from other localities in the Holarctic Region fit into this subgeneric arrangement of Filippova (table II). However, 3 species from the Australian Region and 2 from the Ethiopian Region could not be placed in any of these subgenera listed by Filippova. On the basis of adult characters 2 of the species from the Australian Region had been included in the subgenera *Sternalixodes* Schulze, 1935 (*I. holocyclus*), and *Endopalpiger* Schulze, 1935 (*I. tasmani*). Both of these species in their larval stage showed chaetotaxic features different from those of the other species examined or reported in the literature. These differences appear to confirm the validity of these 2 subgenera as well. The third species from the Australian Region (*I. kohlsi*) was unusual in that considerable variation occurred in the number of setae present as well as in their disposition and it was difficult to establish the exact setal patterns. The subgeneric status of this species must of necessity await the examination of additional specimens. The 2 species from the Ethiopian Region (*I. pilosus* and *I. rubicundus*) showed similar setal patterns for the most part and these possibly represent a new subgenus. In view of the large number of species of *Ixodes* known from tropical Africa, however, it it considered advisable

TABLE II.—*Comparison of the chaetotaxy of 30 species of* Ixodes *larvae.*

	Body (pairs)									Capitulum	
	Sc	Md	Cd	S	St	Pa	Pm	Mv	A	Ph (pairs)	Palp
***Ixodes* s. str.**											
I. ricinus	5	7	4	1	3	2	4	4	1	2	13
I. kazakstani	5	7	4	1	3	2	4	4	1	2	13
I. laguri	5	7	4	1	3	2	4	4	1	2	13
I. redikorzevi	5	7	4	1	3	2	4	4	1	2	13
I. occultus	5	7	4	1	3	2	4	4	1	2	13
I. persulcatus	5	7	4	1	3	2	4	4	1	2	13
I. apronophorus	5	7	4	1	3	2	4	4	1	2	13
I. muris	5	7	4	1	3	2	4	4	1	2	13
I. scapularis	5	7	3	1	3	2	4	4	1	2	13
I. dentatus	5	7	3	1	3	2	4	4	1	2	13
I. bishoppi	5	7	2	1	3	2	4	4	1	2	13
I. cestai	5	7	4	1	3	2	4	4	1	2	13
I. brunneus	5	7	4–6	1	3	3	3–5	3	1	2	13
Pholeoixodes											
I. plumbeus	5	7	2	0	3	2	4	3	1	2	12
I. crenulatus	5	7	2	0	3	2	4	3	1	2	12
I. lagodechiensis	5	7	2	0	3	2	4	3	1	2	12
I. marxi	5	9	2	0	3	2	4	3	1	2	12
I. texanus	5	9	2	0	3	2	4	3	1	2	12
I. cookei	5	8	2	0	3	2	4	4	1	2	12
Ixodiopsis											
I. stromi	5	7	2	0	3	2	4	3	1	2	12
I. angustus	5	8	2	0	3	2	4	3	1	2	12
Eschatocephalus											
I. vespertilionis	5	7	2	0	3	2	4	3	1	2	13
Exopalpiger											
I. trianguliveps	5	6	2	0	3	3	5	3	1	2	12
Scaphixodes											
I. tauricus	5	8	5	0	3	3	7	3	1	2	13
I. berlesei	4	8	2	0	3	—	—	—	—	—	13
Sternalixodes											
I. holocyclus	3	10	5	4	3	2	6	4	1	2	12
Endopalpiger											
I. tasmani	4	8	6	7–8	3	1–2	7	5	1	2	12
Subgenus ?											
I. rubicundus	4	7	4	4	3	3	6	3	1	2	13
I. pilosus	4	8	4	4	3	3	7	3	1	2	13
*I. kohlsi**	4	(——28–34——)			(——33–40——)					2	13

Sc, Scutal; Md, Marginal dorsal; Cd, Central dorsal; S, Supplementary; St, Sternal; Pa, Preanal; Pm, Premarginal; Mv, Marginal ventral; A, Anal; Ph, Posthypostomal.

* Numbers in parenthesis represent the total number of body setae on the dorsal and ventral surfaces respectively.

to await the laboratory rearing of more of these before any more new subgenera are established.

Variation in the number of certain setae appeared to be characteristic of the species in the genus *Ixodes* and this was observed more in the body group and to a lesser extent in the other groups (tables I and II). This variation had also been reported by Glashinskaya-Babenko (1949) and Filippova (1958) for several species of *Ixodes* found in the USSR, but the inclusion of several species in the present study not available to these workers has shown that this range of variation is much greater. The body setae found to vary in number were the supplementary which ranged from 0 to 8 pairs, the marginal dorsal from 6 to 10 pairs, the central dorsal from 2 to 6 pairs, the premarginal from 3 to 7 pairs, the preanal from 1 to 3 pairs, the marginal ventral from 3 to 5 pairs and the scutal from 3 to 5 pairs.

The palpal setae of the capitular group exhibit a small degree of variation among the species of *Ixodes* studied with some species having 12 setae and others having 13 (table II). This feature found application in the subgeneric arrangement of Filippova (1958).

Setal length might be useful in the separation of larval *Ixodes* although its use appears limited. For example, 15 species could be separated into 2 groups when the length of the first 2 marginal dorsal setae on each side is compared to the length of the scutal setae. Those which have marginal dorsal setae 1 and 2 less than twice as

long as the scutal setae are *I. marxi, I. cookei, I. texanus, I. muris, I. angustus, I. rubicundus, I. scapularis, I. pilosus, I. holocyclus, I. tasmani* and *I. kohlsi.* The second group in which marginal dorsal setae 1 and 2 are more than twice as long as the scutal setae includes *I. brunneus, I. dentatus, I. bishoppi* and *I. festai.* This same feature was used by Filippova (1954, 1958) to separate species of larval *Ixodes* in the USSR. This same worker also found that the distance between the post-hypostomal setae would separate *I. ricinus* and *I. persulcatus.* This spatial relationship of setae might possibly have a wider application and should be investigated in relation to other species. Similarly, the nature of setae should be more thoroughly studied because setal differences have been used only on one occasion by Filippova (1958) to separate *I. laguri* and *I. redikorzevi.*

The following key to the species of larval ticks of the genus *Ixodes* in the eastern United States is included here to demonstrate that sufficient characters are now available to separate species of larval ticks on a wide geographical basis. Although keys to species of the world were made to include all of the species examined in this study and adequately described in the literature, it was not considered feasible to publish these keys because of their present limited usefulness.

KEY TO THE SPECIES OF LARVAL TICKS OF THE GENUS *Ixodes* IN THE EASTERN UNITED STATES

1. Dentition 3/3 in anterior third of hypostome. With 7 pairs of marginal dorsal setae. Supplementary setae present. 2
   Dentition 2/2 in anterior third of hypostome. With more than 7 pairs of marginal dorsal setae. Supplementary setae absent. 6
2. With 2 triangular spurs on all coxae. Coxal setae short. Palpi fusiform. .... *I. scapularis*
   Coxal spurs otherwise. Coxal setae long. Palpi not fusiform. 3
3. With 2, 4, or 5 pairs of central dorsal setae. With prominent auriculae. 4
   With 3 pairs of central dorsal setae. Without or with very weak auriculae. ..... *I. dentatus*
4. With 2 pairs of central dorsal setae. Internal spur on coxae I long and slender. Palp longer than tarsus I. ..... *I. bishoppi*
   With 4 or 5 pairs of central dorsal setae. Internal spur on coxae I of medium length and broad. Palp shorter than tarsus I. 5
5. With 3 pairs of preanal setae and 3 pairs of marginal ventral setae. External margin of palp indents sharply near junction of articles 1 and 2. ..... *I. brunneus*
   With 2 pairs of preanal setae, 4 pairs of marginal ventral setae. External margin of palp does not indent sharply near junction of articles 1 and 2. ..... *I. muris*
6. With strong dorsal and ventral projections on palpal article 1. ..... *I. angustus*
   Without strong dorsal and ventral projections on palpal article 1. 7
7. With 4 pairs of marginal ventral setae. With large triangular spur on coxa I; coxae II and III with strong ridge-like spurs. ..... *I. cookei*
   With 3 pairs of marginal ventral setae. With small broadly rounded spur on coxa I; coxae II and III without strong ridge-like spurs. 8
8. With auriculae. Expansion of palpi with internal curvature beginning near the junction of articles 2 and 3. ..... *I. marxi*
   Without auriculae. Expansion of palpi with internal curvature beginning near the junction of articles 1 and 2. ..... *I. texanus*

SUMMARY

The larvae of 56 species of ixodid ticks were examined to determine if chaetotaxy would aid in the identification of both genera and species on a world wide basis. In addition, information concerning 25 species from the USSR was obtained from published records wherein the descriptions and illustrations were considered adequate.

The study of chaetotaxy showed that setae were of definite value for the identification of genera and species. The features having the greatest value include the

number of setae on various parts of the body, the distribution of setae on certain parts of the body, the positional relationship of the setae to certain body structures and to other setae, the length of the setae and the nature of the setae.

Keys to the genera of larval ticks of the world and to the species of larval ticks of the genus *Ixodes* in the eastern United States are included to demonstrate that sufficient characters are now available to separate larvae in a wide geographic area.

ACKNOWLEDGMENTS

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Explanation of Plates

Plate I

Figures 1 and 2. Setal arrangement of *Dermacentor variabilis* (dorsal and ventral view).
Figures 3 and 4. Setal arrangement of *Ixodes bishoppi* (dorsal and ventral view).
Abbreviations. A, anal; Cd, central dorsal; Md, marginal dorsal; Mv, marginal ventral; Pa, preanal; Ph, posthypostomal; Pm, premarginal; S, supplementary; Sc, Scutal; Ss, sensilla sagittiformia; St, sternal.

Plate II

Figure 5. Setal alignment of tarsus I in *Dermacentor, Rhipicephalus, Hyalomma, Boophilus, Margaropus, Anocentor* and *Rhipicentor* (dorsal view).
Figure 6. Setal alignment of tarsus I in *Ixodes* (dorsal view).
Figure 7. Setal alignment of tarsus I in *Amblyomma* (dorsal view).
Figure 8. Setal alignment of tarsus I in *Haemaphysalis* (dorsal view).
Figure 9. Typical setal arrangement of coxa I (ventral view).
Figure 10. Setal alignment on ventral surface of tarsus I.
Figure 11. Setal alignment on dorsal surface of tarsus I.
Figure 12. Setal arrangement on palps.
Abbreviations. Coxae: C_1al, coxa I anterior lateral; C_1mm, coxa I median mesial; C_1pl, coxa posterior I lateral. Tarsus I (ventral): B, basal; M, median; T, terminal. Tarsus I (dorsal): Ha, halleral; Pha, prehalleral; Poh, posthalleral. Palp: Dal, dorsal anterior lateral; Dbl, dorsal basal lateral; Dml, dorsal median lateral; Dmm, dorsal median mesial; Dsm, dorsal submedian; Dst, dorsal subterminal; Dt, dorsal terminal; Vmm, ventral median mesial; Vsm, ventral submedian; Vst, ventral subterminal; Vt, ventral terminal.

PLATE I

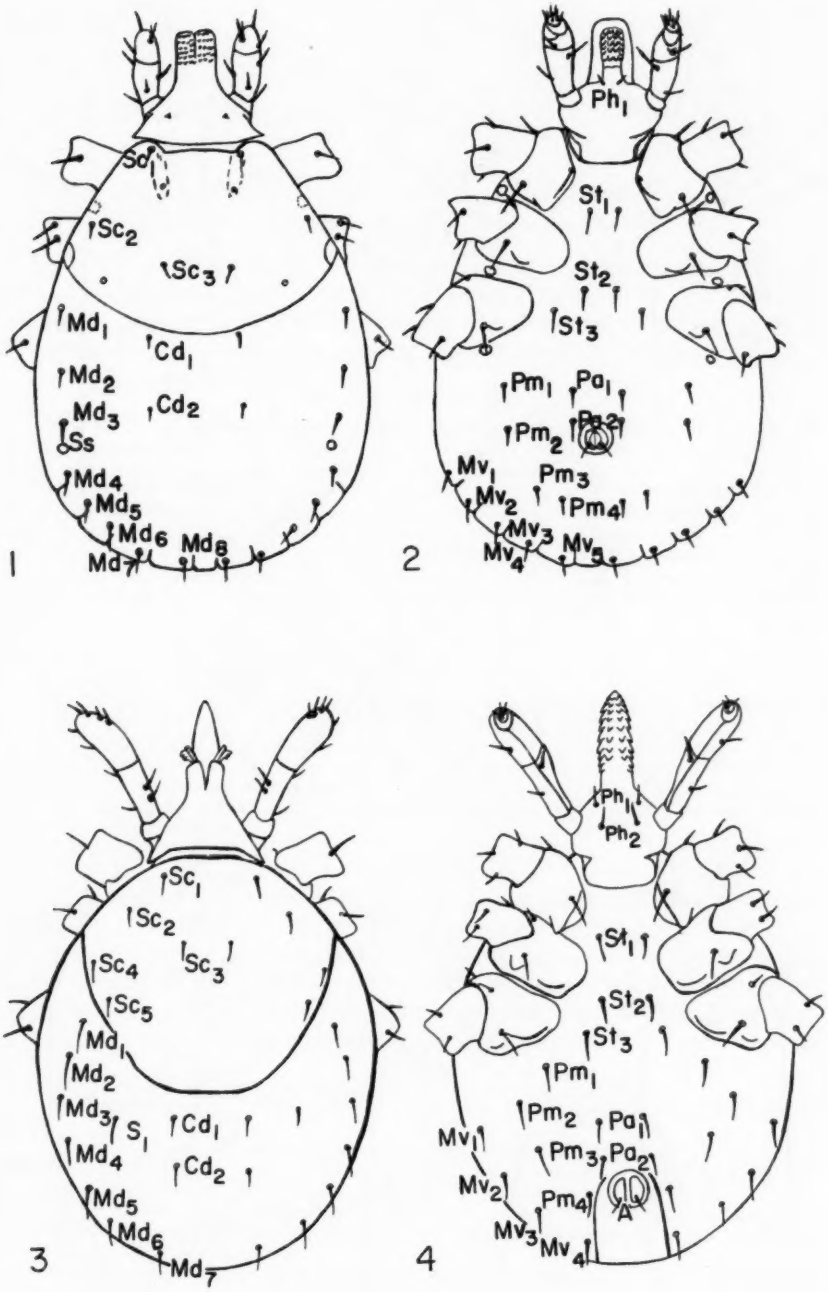
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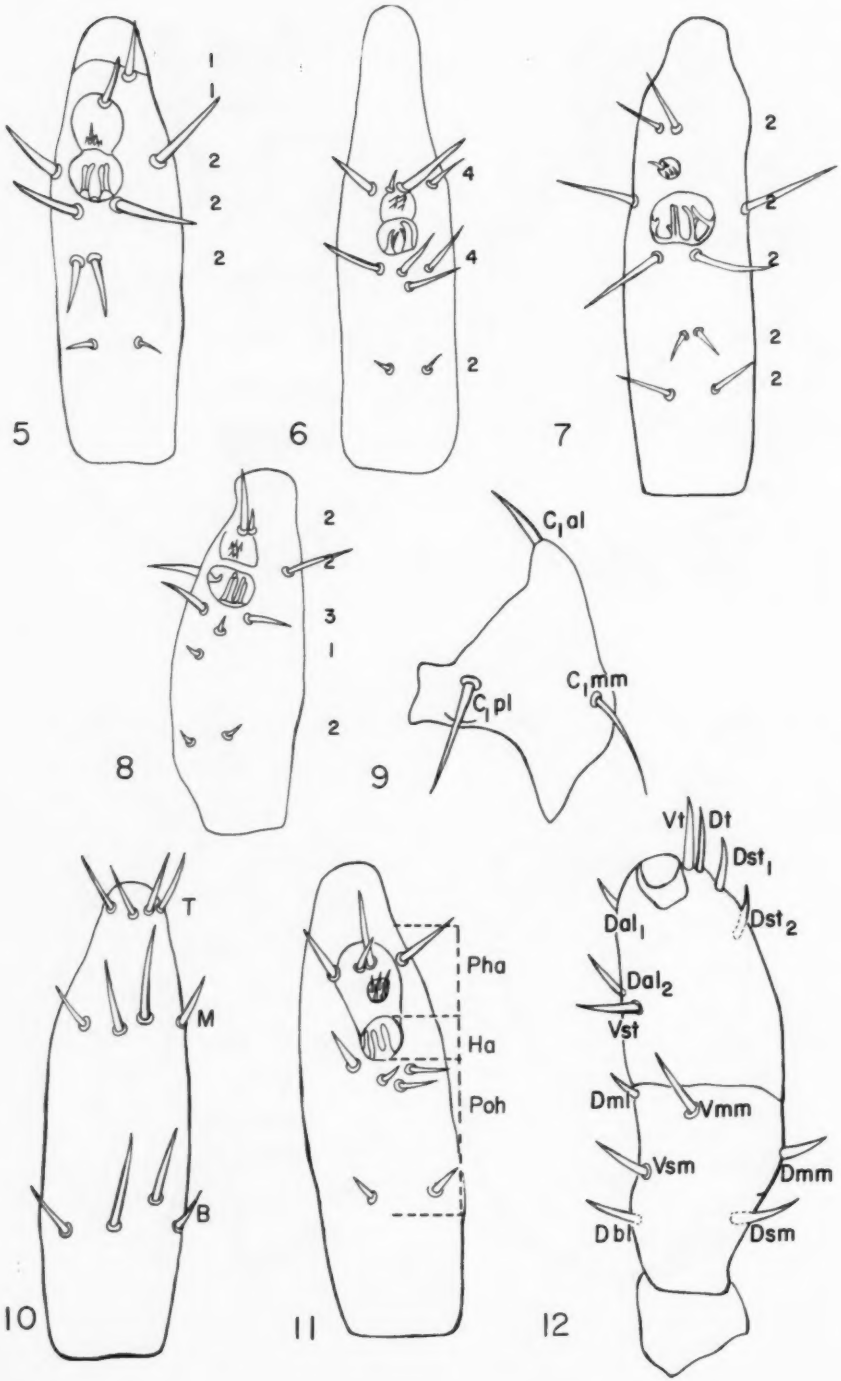
PLATE II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AGE AND INFECTIVITY OF THE FILARIFORM LARVAE OF THE RAT NEMATODE *NIPPOSTRONGYLUS BRASILIENSIS* (TRAVASSOS, 1914)* **

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Haley and Clifford (1958), in a preliminary note, reported that the ability of *Nippostrongylus brasiliensis* to develop to maturity in its normal rat host was influenced by the age of the filariform larvae. The purpose of the present paper is to consider the relationship between larval age and infectivity in greater detail.

MATERIALS AND METHODS

The strain of *N. brasiliensis* used has been maintained in Sprague-Dawley rats for several years. The techniques used to culture the larvae, maintain stock infections, inoculate experimental animals and recover adult worms were the same as those described by Haley (1958). Hardwood charcoal cultures of 24-hour fecal collections were made on the 7th through the 10th days of infection. Stock-infected rats were kept in a screened enclosure during the fecal-collection period in order to avoid contamination of the feces by fly larvae. Cultures in which extensive mold growth occurred were discarded. All cultures were kept in a fly-proof enclosure in an air conditioned room. Room temperatures throughout the period of study ranged from about 22 to 28 C. Under these conditions about 4 days elapsed from the time a culture was made until large numbers of filariform larvae were present on its surface. Therefore the fourth day was taken as zero time in the determination of the age of filariform larvae (e.g. larvae from a culture made 10 days previously were regarded as being 6 days old). This method should be regarded as being accurate within ± 1 day.

Different groups of Sprague-Dawley rats were inoculated intracutaneously with filariform larvae that ranged from 3 to 111 days in age. Intracutaneous inoculation was used instead of the skin penetration method of infection because with the former a much greater degree of precision can be obtained and it is much faster and more convenient than the latter. Male and female rats were used in about equal numbers in most experiments, and host age was controlled so as not to be a factor in the tests. All animals were sacrificed and examined for adult worms on the 10th day of infection.

RESULTS

The development of *N. brasiliensis* in rats inoculated with filariform larvae that ranged in age from 3 to 111 days is shown in table I and figure 1. In many of these tests the larvae used were raised from different stock infections. Several of the experiments summarized in table I, however, were made with larvae that were raised not only from a single stock infection, but raised in the same culture dish. These data have been summarized separately in table II.

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** The first author has recently completed a study of topotypic material of *N. brasiliensis* (Travassos, 1914), and *N. muris* (Yokogawa, 1920). From this study and other evidence it has been concluded that *N. muris* is a synonym of *N. brasiliensis*. This view agrees with that arrived at by Travassos and Darriba (1929) following a study of the literature. A paper summarizing the details of the systematics as well as certain aspects of the biology of *N. brasiliensis* is in preparation by the first author.

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TABLE I. *Adult* N. brasiliensis *burdens in rats 10 days after intracutaneous inoculation with filariform larvae of different ages.*

Age of larvae in days	No. of larvae given per rat	No. of rats inoculated	Age of rats in weeks	Range in percentage recovery	Mean percentage recovery	Standard deviation
3	590	10	12	24.1 – 70.7	55.1	15.9
6	590	10	8	32.8 – 59.3	48.0	10.5
12	570	10	5	26.0 – 66.5	41.8	12.8
12	480	8	12	52.1 – 79.4	62.8	10.2
16	850	10	12	47.0 – 65.5	56.0	6.3
18	580	10	12	37.2 – 67.1	53.8	10.0
21	580	10	6	30.9 – 61.2	49.6	10.2
22	405	8	12	37.8 – 62.7	52.6	8.1
25	535	8	13	31.0 – 68.0	54.2	12.5
27	430	10	10	26.7 – 53.5	42.5	9.3
28	485	11	14	26.8 – 62.4	48.9	14.4
32	625	10	12	25.3 – 58.2	39.8	9.3
35	585	10	8	17.1 – 40.5	28.8	7.9
38	550	12	13	31.1 – 70.0	47.2	11.8
41	410	7	9	14.4 – 28.7	19.9	6.4
44	430	10	9	6.0 – 22.5	13.7	6.2
44	480	10	13	12.1 – 30.6	22.0	7.0
46	450	10	15	33.3 – 64.4	50.2	10.9
52	350	8	10	2.0 – 14.9	9.4	4.1
52	400	5	7	8.5 – 34.2	21.4	12.1
54	340	10	6	0 – 5.3	1.1	1.7
56	435	8	9	19.1 – 48.9	35.8	10.4
64	430	10	8	5.3 – 35.5	15.7	9.4
68	370	7	9	0.8 – 5.4	2.8	1.7
75	390	8	10	0.3 – 9.5	4.9	3.7
87	380	5	10	1.3 – 4.2	2.1	1.4
88	370	10	7	0.3 – 5.1	1.7	1.5
94	365	4	10	0 – 2.2	0.7	1.1
111	400	3	6	0 – 0.5	0.02	0.1

$$\text{Percentage recovery} = \frac{\text{No. of adult worms recovered}}{\text{No. of larvae given}} \times 100.$$

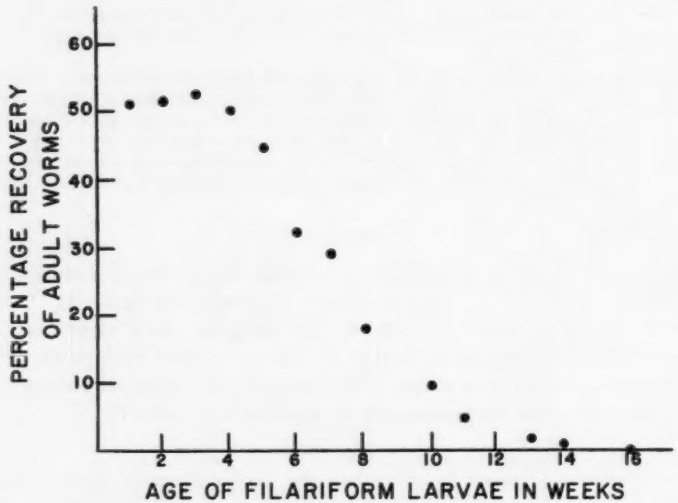

FIGURE 1. Mean percentage recoveries of adult *N. brasiliensis* from rats 10 days after intracutaneous inoculation with filariform larvae of different ages.

TABLE II. *Adult* N. brasiliensus *recoveries from rats 10 days after intracutaneous inoculation with filariform larvae of different ages that were raised in the same culture dish.*

Culture dish no.	Age of larvae in days	No. of larvae given per rat	No. of rats inoculated	Age of rats in weeks	Range in percentage recovery	Mean percentage recovery	Standard deviation
1	6	590	10	8	32.8 – 59.3	48.0	10.5
	27	430	10	10	26.7 – 53.5	42.5	9.3
2	16	850	10	12	47.0 – 65.5	56.0	6.3
	28	485	11	14	26.8 – 62.4	48.9	14.4
3	25	535	8	13	31.0 – 68.0	54.2	12.5
	64	430	10	8	5.3 – 35.5	15.7	9.4
4	32	625	10	12	25.3 – 58.2	39.8	9.3
	56	435	8	9	19.1 – 48.9	35.8	10.4
5	12	480	8	12	51.2 – 79.4	62.8	10.2
	75	390	8	10	0.3 – 9.5	4.9	3.7
6	22	405	8	12	37.8 – 62.7	52.6	8.1
	68	370	7	9	0.8 – 5.4	2.8	1.7
	94	365	4	10	0 – 2.2	0.7	1.1
7	18	580	10	12	37.2 – 67.1	53.8	10.0
	38	550	12	13	31.1 – 70.0	47.2	11.8
	46	450	10	15	33.3 – 64.4	50.2	10.9
	88	370	10	7	0.3 – 5.1	1.7	1.5

DISCUSSION

Under the conditions of the above experiments there was little or no difference in the mean percentage recoveries of adult *N. brasiliensis* from rats inoculated with filariform larvae that ranged from 3 days up to about 4 weeks in age. The 11 groups of rats inoculated with larvae in this age range had mean recoveries of from 41.8 to 62.8 with a grand mean of 51.4 percent. These data are in close agreement with the first author's earlier work in which larvae from 7 to 10 day old cultures were used (Haley, 1958). Thorson (1954), Twohy (1956) and others have obtained similar recoveries using larvae that ranged from 5 to 12 days in age.

Adult worm recoveries from rats inoculated with larvae that were about 4.5 weeks old or older generally showed a decrease as the age of the larvae increased. It is interesting to note, however, that 8 of the 12 groups of rats given larvae that were 32 to 64 days old still had substantial worm recoveries, ranging from about 20 to 50 percent. The six groups of animals inoculated with larvae that were 68 days of age or older had very low adult worm burdens. The group means all were less than 5 percent, and the highest recovery from an individual animal was less than 10 percent. The oldest larvae tested were 111 days in age, and only a few of them were able to develop to maturity in the rat.

Some of the variation in these results undoubtedly was due to the fact that larvae from different stock infections or cultures were used in many of these experiments. This variation between different cultures is illustrated by the data in table II. It should be noted, however, that the data in table II also demonstrate that the general finding of a decreasing larval infectivity with an increasing larval age is evident even in populations of larvae that were raised in the same culture dish.

These results are in general accordance with those obtained in studies of other parasitic nematodes. Payne (1923) claimed that old larvae were less infective than young larvae in experiments with *Necator americanus* in man and *Ancylostoma caninum* in dogs. A decrease in infectivity with increasing age also has been reported for the infective stage of *Ancylostoma* by Rogers (1939), *Haemonchus contortus* by Rogers (1940) and *Ascaridia galli* by Elliott (1954).

A number of factors that may affect larval vigor and survival within a given culture, such as temperature, moisture, larval density, and substrate composition,

need careful study. It would be valuable also to have information on the nature and utilization of reserve food supplies in the filariform larvae of *N. brasiliensis*. Preliminary work by Haley and Clifford (1958) indicated that a large amount of Sudan-stainable material was present in larvae 1 to 4 weeks in age, but that larvae 6 weeks old and older stained weakly or not at all.

SUMMARY

Filariform larvae of *N. brasiliensis* that varied in chronological age from 3 to 111 days were studied with respect to their infectivity for laboratory rats. Infectivity was high and showed little change for larvae that ranged from 3 days up to about 4 weeks in age. Beyond this time there was some marked variation in rate, but ultimately larval infectivity decreased as larval age increased. Some of the larvae that were 111 days in age were still capable of developing to maturity in the rat host, but the percentage development was very small. Tests made with larvae raised in the same culture dish showed the same general trend as that obtained with larvae reared from different stock infections or in different culture dishes.

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EFFECTS OF SUPERINFECTION ON THE BEHAVIOR OF *TOXOCARA CANIS* LARVAE IN MICE*

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Following the identification of the larval stage of *Toxocara canis* in the liver of a child in New Orleans (Beaver et al, 1952), numerous confirmatory case reports have shown that infection with larvae of this ascarid of dogs is an important cause of disease in children. Inasmuch as infection is acquired through ingesting soil previously contaminated by infected dogs, children at the dirt-eating stage of development are most frequently and heavily infected. In most of the temperate and warmer regions of the world *T. canis* infection in dogs is extremely common. It occurs most frequently in young dogs and is more common in the male than in the female adult (Ehrenford, 1957). It has been found prevalent among well-cared-for as well as stray dogs in New Orleans (Vaughn and Jordan, 1960). Infective eggs of *T. canis* have been found abundantly in door-yard soil (Headlee, 1936; Beaver, 1952). These conditions provide opportunities for young children to acquire various degrees of superinfection.

Acquired resistance to nematode infection has been frequently demonstrated. As far as ascarids are concerned, the general conclusion has been that previous infection induces resistance which affects primarily the migration and survival of larvae in the tissues. Studies on the larvae of the pig ascaris have shown that fewer of them can be recovered from the liver and lungs of mice previously infected than from those given equivalent single inoculations (Wagner, 1933); single or multiple sublethal inoculations render guinea pigs resistant to otherwise lethal doses of infective eggs (Kerr, 1938); and in mice immunized by previous infections, the larvae are retarded in their growth and migration, remaining for longer periods in the liver before migrating to the lungs (Sprent and Chen, 1949). Comparative studies on the larval migration patterns of various ascarids have shown that *Toxocara canis* differs fundamentally from *Ascaris lumbricoides* in that its larvae are adapted to paratenic transfer, i.e., passage through a succession of intermediate cannibal or predator hosts after having reached infectivity for the final host (Sprent, 1952); therefore, the larvae of these 2 species may be affected differently by superinfection.

The present study of *T. canis* was undertaken to determine the effect of superinfection on the number, distribution and encapsulation of larvae that enter and persist in the tissues.

MATERIALS AND METHODS

Eggs were obtained directly from the uteri of adult worms and were incubated at room

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temperature in 1 percent formalin. When infective, the eggs were washed free from formalin and inoculated by stomach tube into lightly ether-anesthetized mice.

Sprent's method (1952), slightly modified, was used for the recovery of larvae from tissues. The time of examination was at least 2 weeks after inoculation at which time many of the larvae had become encapsulated. For determining the extent of encapsulation, both pepsin, which digests the fibrous capsule around encapsulated larvae, and trypsin, which does not, were used. The tissues were first digested in trypsin. The emulsion was strained through a double layer of gauze and the larvae recovered from the filtrate were considered as free larvae. The encapsulated larvae, retained in the gauze were washed and further digested in pepsin. The number of those larvae obtained from the 2d digestion reflected roughly the extent to which encapsulation had occurred in the tissues. Squash preparations were most suitable for counting larvae in the brain.

In a preliminary experiment it was found that the average lethal dose of infective *T. canis* eggs is somewhat above 2,000 per 20- to 30-gram mouse. Therefore, a total inoculum of 2,000 eggs per mouse was used in the superinfection experiments. Altogether, the experiment involved the use of 6 groups of mice. It was started with 5 groups designated A, B, C, D (5 mice in each), and E (10 mice). In the first 4 groups, each mouse was given 2,000 infective eggs in 3, 4, 5, or 6 divided doses respectively, evenly spaced but in increasing numbers over a period of 6 weeks (table I). In E, the 1st control group, each mouse received 2,000 infective eggs in

TABLE I. *The average number and distribution of* Toxocara canis *larvae in the tissues of 4 groups of mice given 2,000 infective eggs in 3, 4, 5 or 6 divided doses spaced over a period of 6 weeks and sacrificed 8 weeks after the first inoculation. Of the 2 control groups, 1 (E) was given the same total inoculum in 1 dose and sacrificed 8 weeks later, the other (F) was given 1,000 infective eggs in 1 dose and sacrificed 2 weeks later.*

Host group	Number of animals	Number of inoculations*	Average number of larvae recovered from brain	lung	liver	carcass	total
A	5	6	129	22	382	466	999
B	5	5	117	15	295	497	924
C	5	4	109	14	291	428	842
D	5	3	96	22	377	400	895
E	5	1	220	5	43	1051	1319
F	4	1	67	10	88	454	619

* Numbers of infective eggs given were as follows: Group A, 100, 150, 200, 250, 300, and 1000; group B, 100, 200, 300, 400, and 1000; group C, 200, 300, 500, and 1000; group D, 500, 500, and 1000.

a single inoculation. Of the 7 survivors at 8 weeks of infection in this group, 5 were taken at random for examination. The mice of the 4 reinfected groups were examined beginning 2 weeks after the final inoculation. In a 2d control group (F) of 4 mice, each was given 1,000 larvae in a single dose and sacrificed 2 weeks later.

RESULTS

1. *Effects of Infection on the Host*

All of the 20 mice in the superinfected groups survived the infection for the period of the experiment. Among the 10 control mice each of which received in a single dose the same number of eggs as the superinfected test mice (2,000), there were 3 deaths from typical verminous pneumonia 7, 10 and 15 days after inoculation.

Occasional hemorrhagic areas were found in the liver of both singly and repeatedly infected mice. In most cases, yellow-white nodules measuring up to 5 mm in diameter were seen scattered throughout the organ. More nodules of this type were observed in superinfected mice than in the controls. In the lungs of mice given either type of infection, hemorrhagic areas were invariably present. However, the degree of hemorrhage varied considerably in both singly and multiply infected mice. Occasionally yellow-white nodules were seen scattered in the lungs, although they were always very much less numerous than in the liver. The kidneys were particularly severely affected as indicated by the yellowish-white, diffuse lesions over the entire surface in both types of infections. The nodules were seldom discrete.

In the brain, small areas of hemorrhage were seen in 2 mice.

By tissue-press preparation, it was revealed that the yellow-white nodules observed always in the muscles, liver and kidneys, and less frequently in the lungs, were fibrous capsules containing 1 or rarely 2 motile larvae. The organs in which encapsulations were seen most abundantly on gross examination were the liver and the skeletal muscles. Although larvae were numerous in the brain, encapsulation was never observed in that organ.

2. Number and Distribution of Larvae in the Tissues

In the superinfected groups the larvae were found in greatest numbers in the carcass (table I). Next in order came the liver, brain and lungs. Although the average total yields were fairly constant, there was considerable variation in the numbers recovered from the different locations.

In the 1st control group most of the larvae were recovered from the carcass, followed in order by brain, liver and lungs. In the 2d control group, the order of decreasing number of larvae in tissues was the carcass, liver, brain and lungs. In both control groups the number of larvae in the liver was markedly less than in the carcass (table I).

When the ranges and means of the percentage of inoculated larvae recovered from various tissues for each group were computed, it became evident that there were close similarities in the total yields and in the distribution of larvae from various tissues among the superinfected groups. On the other hand, the total yields differed considerably between the superinfected and the control groups (table II).

TABLE II. *The percentage of inoculated* Toxocara canis *larvae recovered from various locations in mice given a total of 2,000 infective eggs in 1 to 6 doses spaced over 6 weeks and sacrificed 8 weeks after the initial dose, or (in group F) given 1,000 larvae and sacrificed 2 weeks later.*

Group	Number of		Average percentage of inoculated larvae recovered from									
	mice	inoculations	brain		lung		liver		carcass		all tissues	
			Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
A	5	6	4– 9	6	<1–1	1.0	11–34	19	12–31	23	42–60	49.8
B	5	5	4– 9	6	<1–1	0.7	8–20	15	22–27	25	43–49	46.2
C	5	4	2– 9	5	<1–1	0.7	4–29	15	17–26	21	37–51	42.2
D	5	3	3– 7	5	<1–2	1.0	12–28	19	16–25	20	40–45	45.0
E	5	1	8–15	11	<1–1	0.3	1– 5	2	39–65	53	48–84	65.8
F	4	1	3–14	7	0–2	1.0	4–13	9	38–51	45	51–77	61.9

The controls had about 20 percent more of the inoculated larvae in their tissues than the superinfected groups, and a remarkably larger proportion of them was found in the liver of superinfected mice than in the controls. In both groups very few larvae were recovered from the lungs. The percentage of larvae in the liver was higher in the 2d control group than in the 1st, but the difference in the percentage of inoculated larvae recovered collectively from all tissues of the 2 control groups was not significant.

3. Extent of Encapsulation

There were similarities in the percentages of encapsulation in the liver and carcass respectively in the superinfected and singly infected animals (Table III). However, the rate of encapsulation in both organs was higher in the singly inoculated animals. Although the percentage of encapsulation in the liver was relatively

TABLE III. *The extent of encapsulation of* Toxocara canis *larvae in the liver and carcass of mice given 2,000 larvae in single or repeated inoculations and sacrificed 8 weeks after initial inoculation.*

Organ	Inoculation	No. of mice	Larvae recovered per mouse		
			total	encapsulated	
				no	percent
Liver	Repeated	20	336	214	64
	Single	5	42	33	78
Carcass	Repeated	20	447	295	66
	Single	5	1051	823	78

low in the superinfected animals, the average number of encapsulated larvae was extremely high owing to their great concentration in that organ.

DISCUSSION

All of the mice that were repeatedly inoculated with increasing numbers of infective *T. canis* eggs, to a total of 2,000, survived at least 2 months; whereas, among the controls which were given equivalent inocula in 1 dose, there were 3 deaths among 10 mice within 2 weeks after inoculation. One of the methods used in the study of immunity to ascaris infection in rodents is to compare the rates of survival after inoculations of a computed lethal dose of eggs in animals "immunized" by previous sublethal inoculations (Kerr, 1938). The results of the present study suggest that it is possible by repeated inoculation of sublethal doses to induce a tolerance to *T. canis* infection. In the case of pig ascaris in guinea pigs the induced resistance decreases sharply after the larvae have disappeared from the body of the host, suggesting that maintenance of immunity depends on the presence of the parasites (Kerr, 1938). In animals other than the final host, the larvae of *T. canis* tend to remain in the tissues, free or encapsulated, for very long periods. Therefore, until *T. canis* can be removed from the tissues, it will not be possible to determine the degree of persistence of the immunity induced by them.

Comparison of the total yields of larvae obtained from single and repeated infections indicates clearly that in the latter there is a considerable loss of larvae. The degree of loss is not correlated with the number of inoculations received by the animals, as the total larval counts are fairly constant among the 4 groups of animals reinoculated at different time intervals. Statistical analysis using Student "t" test for comparison of reinoculated groups and the controls indicates that the difference in total larval recovery is highly significant (P, 0.001). At present no satisfactory explanation has been found for the consistently lower recovery from the test animals (42 to 50 percent) as compared with the 2 control groups (62 and 66 percent). The comparison of total larval counts between single infection and superinfection suggests that repeated inoculation of *T. canis* in mice induces a degree of resistance which certainly is detectable by its effect on the number of larvae that can be recovered from the hosts' tissues. However, there is no evident correlation between the degree of resistance, in terms of the number of larvae present, and the number of reinoculations the animals received.

Superinfection with *T. canis* also affects the distribution of larvae in the various tissues. One of the most remarkable findings was the high proportion of larvae recovered from the liver which accounted for one-third or more of the total yields. The large number of scattered pale nodules observed on the liver of superinfected

mice further indicates that more larvae were encapsulated in this organ. Kerr's (1938) finding of the walling-off and destruction of pig ascaris larvae in the liver of reinfected guinea pigs indicated that this organ was a very important barrier to the migration of the larvae to the lungs. In immunological studies in mice infected with pig ascaris, Sprent and Chen (1949) found that during initial infection the "liver ratio," i.e., the number of larvae in the liver divided by the total number in the liver and lungs, was high for 5 days and decreased to a low level by the 8th day due to the migration of larvae from the liver to the lungs. Contrariwise, in mice immunized by repeated infections the "liver ratio" continued to be high because the larvae did not migrate to the lungs and tended to become rapidly encapsulated. In the present study no larvae were found in the intestinal wall either after single or repeated inoculations. Furthermore, the relatively great abundance of larvae in the liver of superinfected mice suggests that the mechanism of immunity does not operate in the intestine. From this observation together with the findings reviewed above it appears likely that the most effective immunological response takes place in the liver. The question of whether in relatively immune or resistant animals the larvae fail to penetrate the intestinal wall, or perhaps fail even to hatch from the eggs at all was considered. Examinations of the feces collected for 48 hours after the last of the repeated inoculations showed that the number of unhatched or free larvae eliminated from the intestine was negligible.

The present study and others (Smith and Beaver, 1953; Sprent, 1955) indicate that the larvae of *T. canis* tend somewhat to accumulate in the brain where they remain free for long periods and seldom cause grossly observable lesions. It is noteworthy that in the superinfected mice relatively few of the larvae were found in the brain. This is reasonable in that if a high proportion of them are trapped in the liver fewer will succeed in migrating elsewhere.

The percentages of encapsulation in both the liver and skeletal muscles are lower in superinfected mice than in control mice. In the latter the period of infection was either 2 months or 2 weeks, while in the test animals the inoculations were spread from 2 months to 2 weeks prior to examination. Although the percentage of encapsulation was lower in superinfected mice, the number of encapsulated larvae in the liver was about 7 times greater than in the same organ of the controls. Thus, while superinfection did not affect the frequency of encapsulation, it did affect the number of encapsulated larvae located in the liver. It appears therefore that encapsulation does not destroy the larvae. It has been suggested that it may instead protect them (Beaver, 1956). At present, there is no explanation for the apparent loss of larvae in the superinfected animals.

SUMMARY

Each mouse in 4 groups was infected with a total of 2,000 *Toxocara canis* larvae given in 3, 4, 5, or 6 spaced inoculations over a period of 6 weeks and sacrificed 8 weeks after the 1st inoculation. In 2 control groups, mice were given respectively 2,000 and 1,000 larvae in single inoculations. The 1st was sacrificed 8 weeks, the second, 2 weeks after inoculation. While all of the test animals survived, 3 in the 1st control group died. Pepsin and trypsin digests were used for the recovery of larvae from the tissues. The order of decreasing numbers of larvae recovered from various locations was the carcass, liver, brain and lungs for the superinfected and

2-weeks control groups, whereas, in the 8-weeks control group the order was the carcass, brain, liver and lungs. In the superinfected mice there was about 20 percent lower recovery of the inoculated larvae than in either of the control groups. Superinfection had no apparent effect on the extent of encapsulation but did increase the relative number of encapsulated larvae located in the liver.

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THE ROLE OF RATS AND MICE IN THE TRANSMISSION OF THE PORKWORM, *TRICHINELLA SPIRALIS* (OWENS, 1835) RAILLETT, 1895*

HARRY A. ROBINSON AND O. WILFORD OLSEN**

Transmission of trichina by means of fecal material containing larvae and cysts from animals which have eaten a meal of trichinous meat has been demonstrated by several authors (Salzer, 1916; McCoy, 1938; Spindler, 1953). Others, however, consider the role of feces from animals which have eaten trichinous meat insignificant as a means of transmitting *Trichinella* (Lintz, 1916; Raffensperger, 1918; Hall, 1937; Kolbe, 1942; Gould, 1943).

Hemmert-Halswick and Bugge (1934) concluded that young intestinal trichinae which have not molted are capable of resisting gastric digestion by virtue of having a cuticle and such parasites, if present in the stool within a day or two after the infection, should, therefore, be able to produce infection if eaten by other animals. These workers recovered free trichinae in the stools of rats 5 hours after infection, many dead trichinae after 24 hours, and only an occasional one by the 10th day. They found none after the third week of continuous examination of feces of infected rats. Catron (1937), testing for possible intra-uterine infection, kept young rats with their mothers which were heavily infected with trichinae. He claimed that no infections were transmitted to the young from either the feces or milk of the infected mother.

Jacob (1941) included that, although Germany was under rigid trichinosis control, the disease still prevailed due to the general appetite of dogs and pigs for the feces of other animals. He pointed to experiments which show that fecal ingestion occurs and that pigs may become infected by contamination of their food with feces containing parasites. Kolbe (1942) likewise suggested the possibility of pigs becoming infected from eating the feces of other animals.

Keller (1941) fed trichinous meat to chickens, collected the feces passed by birds during the next two 24-hour periods, and fed the feces to 4 rats and 3 mice, and to 2 rats and 3 mice, respectively. Only 1 rat which was fed feces collected during the first 24-hour period become infected. This was considered insignificant.

Kocylowski (1945) conducted experiments on the role of freshwater fishes in the dissemination of *Trichinella*. He found that carp and pike, after ingesting trichinous meat, passed the parasites in the feces at the end of 4 and 5 days. He found no encysted larvae in the striated muscles, and he made no tests on the viability of the trichinae found in the feces.

Zimmermann, Schwarte, and Biester (1956) used foxes as donor animals, and

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rats and foxes as receptor animals for trichinae. They fed trichinous meat to 9 animals and fed the feces from these animals to trichina-free animals but did not produce infections in the latter. The conclusions of these investigators did not rule out the possibility of infectious fecal material playing a role in the transmission of *Trichinella*.

Methods and Materials

The original course of *Trichinella* for this study was 472 Norway rats collected at the city dump of Fort Collins, Colorado. Seventy-two Sprague Dawley and 342 Wistar strain white rats, 400 white mice, and 10 young Duroc pigs were used in the experiments.

Infectivity of trichinae found in the feces of rats and mice which had eaten a meal of trichinous meat was determined by feeding the encysted, sheathed, and exsheathed larvae to trichina-free rats. Thirty days after feeding, receptor animals were killed and examined for *Trichinella*.

At intervals of 30 days, flesh of wild rats containing trichinae cysts was fed in single feedings to white mice and rats; each mouse received flesh containing 300 cysts and each rat received flesh containing 1,500 cysts at a feeding. Only the feces passed after the first two feedings were used in tests.

Feces collected from 20 mice and 20 rats for 96 hours following the first and second meal were examined for the presence of trichinae.

Free and encysted larvae in feces exposed to moist and dry conditions were tested for survival by feeding them to receptor animals. Biopsies were made to determine whether the experimental animals were free of trichina prior to feeding of cysts and larvae recovered in feces.

The food of experimental mice, rats and pigs was autoclaved at 225 F for 25 minutes as further assurance against accidental infections by trichinae through contamination. Supplements of fresh vegetables were fed to replace vitamins lost through autoclaving. The young of experimental mice and rats were reared in a trichina-free environment.

The entire carcass of each rat and mouse or the diaphragm and tongue of each pig was ground in a food chopper and digested in a standard solution of pepsin-hydrochloric acid-water for 14 hours at 37 C. The undigested material was washed and sedimented for the determination of whether or not parasites were present.

Trichinae cysts and larvae removed from the first 5 fecal pellets passed by rats following a meal of trichinous meat were placed in water and observed for activity. The cysts were exposed to dry and moist conditions, respectively, for intervals of 2 and 3 days. The cysts were digested in artificial digestive juices at the end of each interval to determine whether the larvae were alive.

Results

Of 472 Norway rats collected from the city dump at Fort Collins, Colorado, for infective material, 17.8 percent were infected with *Trichinella spiralis*.

Recovery of Trichinae in Feces

Rats. From approximately 30,000 encysted larvae fed initially to 20 white rats in the amount of 1,500 per rat, 729 free and 41 encysted larvae were recovered in the rats' feces during the first 96 hours after the feeding. This represented approximately 2.6 percent of the total number of cysts eaten. One rat failed to pass trichinae in the feces. During the first 12-hour period (following the original meal of trichinous meat), 371 free and 41 encysted larvae were recovered. This represents 1.4 percent of the cysts fed and approximately 50 percent of those passed. The number of larvae recovered ranged from 0 to 87 with an average of 38.5 ± 23.0 per rat. Thirty-seven cysts were recovered from 1 rat and 3 from 2 rats during this period.

From the same rats, when each was fed a second group of 1,500 cysts each 26 to 30 days later, a total of 6,674 larvae and 15 cysts, or 22.3 percent of the total fed, were recovered. During the first 12-hour period of fecal collection, a total of 3,061 larvae and 15 cysts, or 10.5 percent of the total number fed, was recovered.

The number of larvae and cysts recovered ranged from 83 to 1,383 per rat with a mean of 334.4 ± 121.0.

More free larvae than cysts were passed following each meal of trichinous meat. The greatest number appeared during the first 12-hour period, with diminishing numbers appearing in each succeeding period up to 96 hours. The percentage of larvae recovered after the second meal of trichinous meat was always greater than after the first.

Mice. Each of 20 mice received approximately 300 trichinae cysts when fed the first meal of trichinous meat. Of the total of 6,000 cysts fed to the mice, 389 larvae, ranging from 6 to 33 with a mean of 19.5 ± 6.9 per animal, were recovered in the feces during the 96-hour period after the feeding of the meat. This represented approximately 6.5 percent of the cysts fed. During the first 12-hour period, 187 larvae, or 3.1 percent, were recovered. No cysts were seen (or recovered) in the feces throughout the 96-hour period.

From the second and similar meal of trichinous meat fed to the same mice approximately 30 days later, 755 free larvae, ranging in number from 24 to 75 per mouse with an average of 37.8 ± 11.1 were recovered in the feces during the 96 hours following the meal. A total of 458 free larvae, representing 7.6 percent of the total number fed, was recovered during the first 12-hour period. No cysts were found in the feces during the first 96-hour period following the second feeding of trichinous meat.

Transmission of Trichina to Rats, Mice and Pigs through Infected feces

Rat to rat. Feces from each of 10 rats fed for the first time a meal of trichinous meat were collected for a period of 48 hours on individual trays and allowed to dry there for 4 to 8 hours at room temperature prior to being fed to uninfected rats. At autopsy, 1 to 20 rats fed dried feces was found to be infected with trichinae.

Dried feces collected for 48 hours after the second trichinous meal and fed to 20 rats resulted in 3 infections (fig. 1).

Feces from rats fed the first meal of trichinous meat were collected in trays containing water. When fed to 40 rats, 4 infections resulted. Moist feces from rats which had eaten a second meal 26 to 30 days later produced 18 infections in 40 rats (fig. 1).

A total of 26 of 120 rats became infected by eating dried and moistened feces from rats which had eaten the first, and 26 to 30 days later, a second meal of trichinous meat.

Mouse to mouse. Dry feces obtained from mice having had the first meal of trichinous meat and fed to 30 trichina-free mice resulted in 1 infection. Dry feces from the second meal 26 to 30 days later produced 2 infections in 30 mice (fig. 1).

Feces from the first meal of trichinous meat collected in water for 48 hours produced 2 infections in 30 mice. Those from the second meal resulted in 9 infections in 30 mice (fig. 1).

A total of 14 of 60 mice became infected from dried and moistened feces from mice which had eaten a first, and 26 to 30 days later, a second meal of trichinous meat.

Rat to swine. Feces collected in water for 48 hours from 4 rats had eaten the first meal of trichinous meat infected both the trichina-free pigs to which they had

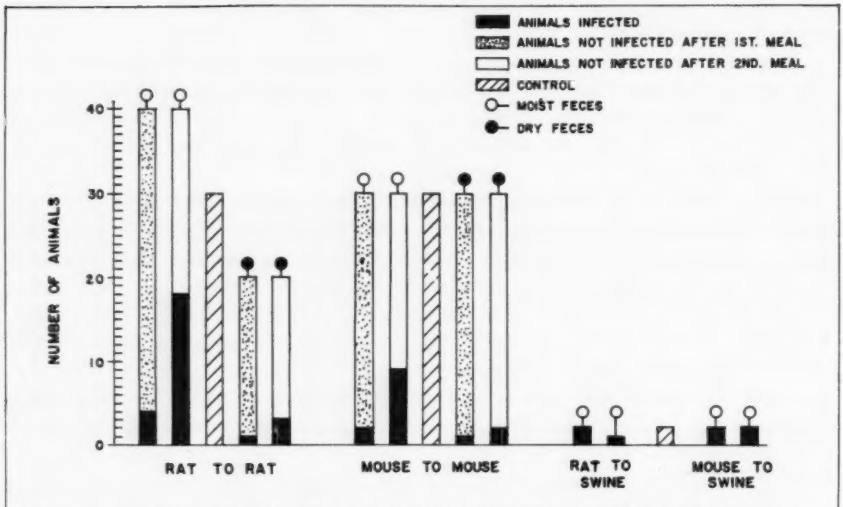

FIG. 1. ANIMALS INFECTED WHEN FED MOIST AND DRIED FECES FROM ANIMALS WHICH HAD EATEN THE FIRST AND SECOND MEAL OF TRICHINOUS MEAT

been fed. Moistened feces from a second meal were fed to a single pig and resulted in an infection (fig. 1).

Mice to swine. Moist feces from 4 mice which had eaten the first meal of trichinous meat produced infections in both pigs to which they had been fed. The moistened feces from 4 mice which had eaten the second meal of trichinous meat resulted in infections in both experimental pigs (fig. 1).

None of the controls used in the experiments was infected.

Infective Stages of Trichinae Found in Feces

Cysts, active sheathed larvae, active exsheathed larvae and dead larvae were recovered in feces of rats that ate trichinous meat. All of these stages were fed to trichina-free rats.

Each of 3 rats became infected when fed bits of undigested meat containing trichina cysts recovered from the feces of rats which had eaten trichinous meat. Another group of 3 rats became infected after they were fed active sheathed larvae. None of the rats fed exsheathed larvae became infected. None of the trichinae were beyond the larval stage when fed to the rats. All rat controls were free of trichinae.

Longevity of Trichina in Feces and Infected Meat

Survival of free larvae in feces. The first 5 fecal pellets passed by each of 60 previously infected rats fed 900 cysts each were used to ascertain the longevity of survival of the larvae in feces. The pellets from 30 rats were exposed to dry conditions at room temperature and those from the other 30 rats to moist conditions for 10 minutes, 1, 2, 3, and 4 hours. The results were calculated on a pooled basis in order of the appearance of the pellets. Viability of the larvae was determined by their movement and coloration and condition of the protoplasm.

In the drying experiment, a total of 180 larvae was recovered from the first fecal pellets passed. When the pellets were exposed to the dry air for approximately 10 minutes, 132, or 73 percent, were active. The total number from the second pellet passed by 30 rats was 144 of which 5, or 3 percent, were active after 1 hour. From the third, fourth, and fifth pellets, 174, 99, and 80 larvae respectively, were recovered. After the drying of the feces for 2, 3, and 4 hours, respectively, no active larvae were found.

In the second part of the experiment, fecal pellets were treated in the same manner as the above except for their being kept moist. Of 197 larvae recovered in the first fecal pellets passed from each of 30 rats and kept in water for 10 minutes, 144, or 73.0 percent, were active; from the second fecal pellets kept in water 1 hour, 176 larvae were recovered and 47, or 26.7 percent, were active. From the third fecal pellets kept in water for 2 hours, 147 larvae were found and 30, or 26.0 percent, were active. From the fourth fecal pellets kept in water 3 hours, 149 larvae were found and 23, or 15 percent, were active. Of 136 larvae recovered from the fifth fecal pellets kept in water for 4 hours, 3, or 2.0 percent, were active.

Cysts in feces. Seventy-two trichina cysts in bits of undigested meat were removed from feces of rats having eaten trichinous meat. Thirty-six of these cysts were placed on a dry tray at room temperature. Four cysts were removed every 2d day through the 18th day. Each group of the cysts when digested in artificial juice on the second, fourth and sixth day produced active larvae. Of the 4 cysts digested on the eighth day, only 2 produced active larvae. No active larvae were seen in tests made on cysts which had undergone this treatment longer than 8 days.

Thirty-six cysts were placed in a Petri dish of water and examined at intervals of 3 days. All of the cysts, when removed in groups of 4 each on the 3rd, 6th, 9th, and 12th days later and placed in artificial digestive juices, were found to contain active larvae. Two live larvae were observed in groups removed on the 15th and 18th days and 1 was found in each of the groups tested on the 21st, 24th and 27th days.

DISCUSSION

Trichinae have been transmitted to dogs through human excreta, to swine through rat feces and to rats through larvae in rat feces (Salzer, 1916; McCoy, 1938; Spindler, 1953). This shows that viable infective larvae and/or cysts are passed in fecal material of animals having eaten trichinous meat, and that they are capable of establishing infections. However, the commonly recognized source of infection is infected flesh. *Trichinella* larvae have been found in the muscles of mammals and birds, and in the intestinal contents of frogs, tadpoles, and necrophagous beetles. It has been suggested that fishes and amphipods may play an important role in disseminating the parasite through the contents of their intestines to omnivorous and carnivorous animals (fig. 2). This suggests a broad range of potential hosts and a complexity of the biology of the parasite, thus making control of the disease equally involved.

Cysts and larvae of *Trichinella* are passed in the feces of rats and mice which have eaten trichinous meat. The majority of them are passed as free larvae in the feces or as cysts in undigested bits of meat during the first 24-hour period subsequent to eating infected meat. Free cysts containing larvae are rarely found.

The condition of larvae in the feces vary when the latter are exposed to dry or

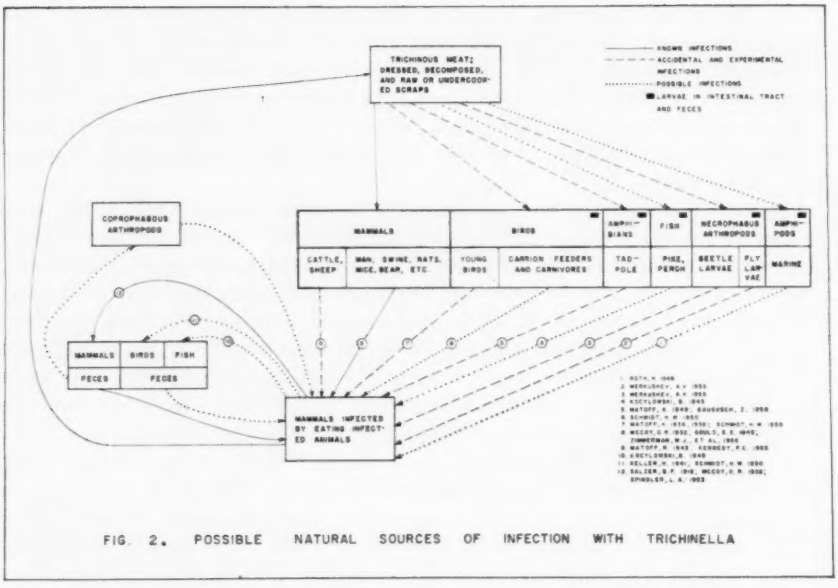

FIG. 2. POSSIBLE NATURAL SOURCES OF INFECTION WITH TRICHINELLA

moist conditions. Larvae in the fecal pellets containing them are completely destroyed when exposed to 2 hours of drying. On the other hand, larvae survived 4 hours in feces kept moist. Thus the longer the feces containing larvae remain outside of the host, the less likely are the possibilities of a trichina infection. Only trichinae within the sheaths or cysts can withstand conditions outside the body for any extended period. Exsheathed larvae are extremely short-lived in the external environment. The fact that larvae are able to survive any period in feces suggests that they constitute a potential source of infection to coprophagus animals such as swine, dogs, and rodents.

The majority of cysts recovered in this study were in bits of undigested meat passed in the feces during the first 24-hour period subsequent to eating the infected flesh, few appearing later. Cysts recovered in the feces and fed to trichina-free rats produced infections.

The number of larvae recovered from the feces following the first meal of trichinous meat ranged from 0 to 11.3 percent of the total cysts fed and 5.2 to 92.2 percent following a second meal 26 to 30 days later. McCoy (1938) reported a comparable condition.

It has been suggested that sheathed larvae found in feces may be able to resist the digestive action and become established in the host. In our study, only the rats fed cysts and sheathed larvae recovered in the feces became infected. Unsheathed larvae failed to establish infections in susceptible experimental animals.

Spindler (1953) stated that feces passed by rats for 28 days after eating trichinous meat were capable of infecting swine. It is difficult to understand the mechanism whereby this could occur under well-controlled experiments.

Rats and mice with ample palatable food are reluctant to eat feces, but under conditions of adversity or starvation there is a tendency toward coprophagy. Catron (1937) kept rats infected with trichinae and uninfected ones to ascertain if infec-

tions might occur in the control animals. He concluded that under normal conditions infections through coprophagy does not occur.

The role of feces in transmitting trichina appears limited. The probability, however, of infections occurring from this source is always present. In man and animals, such infections probably should be light and subclinical in nature, as the number of infective larvae ingested with the feces would be few. The degree of infection, whether light or heavy, however, presents a danger not only to the infected but to the uninfected as well.

It has been suggested that rats are not important in transmitting trichinae to swine, since pigs normally do not eat them. The feces of rats that eat trichinous meat contain infective larvae and are a source of infection to any animal that ingests them. Contamination of the feed of swine with the feces of rats and mice containing larvae would be a source of infection to pigs which in turn may infect humans utilizing such pork for food. However, many adversities may figure in the completion of such infections, the fact remains that they can occur. The extent to which they actually happen is unknown.

Examination of swine fed grain revealed that 0.91 percent of them in the United States (Schwartz, 1938) and 1 percent in Canada (Cameron, 1940) are infected with trichinae. The source of these infections was unknown. The environment of a piggery attracts rats and mice as well as other animals whose feces may contain trichinae and thereby contaminate the feed of the swine, resulting in their infection.*

Schwartz (1938), pointed out that where infections are less than 1 larvae per g of body weight, the use of the compression method is highly inadequate for the detection of the parasites. In our study, 17 of 40 infected animals had less than 1 larvae per g of body weight. Wolffhügel (1938) stated that in order to be sure that the animals may or may not be infected with trichina, the whole musculature must be digested artificially and the sediment examined. Complete carcasses of rats and mice were digested in this experiment.

The danger of transmission of *T. spiralis* through infected feces of rats and mice seems to increase the scope of the parasite. As stated above, this may be the source of infection that occurs in swine fed entirely on grain or cooked garbage or a combination of these. However, this cannot be restricted to rats and mice for many wild mammals ingest infected meat and in the course of their movements may pass trichinae in their feces which in turn may be ingested by susceptible animals, swine and man included (Salzer, 1916; McCoy, 1938; Spindler, 1953).

The results of this and other studies on the biology of trichina indicate that transmission of the parasite is not a simple matter. Any plan for controlling the parasite must take into consideration the numerous ramifications in which the feces of recently infected rats and mice are but a single facet. Due to the complexity of the life history, it is almost impossible to completely eliminate the parasite. Control programs should take into consideration improved sanitary feeding and storage conditions to reduce the spread of the disease. The fact that feces can be a source of infection suggests that sanitary measures are more important than heretofore recognized in the control of this parasite.

* After this manuscript had been prepared, Zimmermann, Schwarte, and Biester (1959, J. Parasit. **45**: 441–445) showed that swine became infected from eating feces of foxes fed trichinous meat.

SUMMARY

1. Approximately 17.8 percent of 472 Norway rats collected from the city dump of Fort Collins, Colorado, were found to be infected with trichinae.

2. One of 30 mice was infected when fed dried feces from other mice appearing during the first 48 hours following the first meal of trichinous meat; whereas, 2 of 30 mice were infected when fed dried feces from other mice resulting from the second trichinous meal.

3. One of 20 rats was infected when fed dried feces from other rats appearing during the first 48 hours following the first meal of trichinous meat, while 3 of 20 rats were infected when fed dried feces from other rats resulting from the second trichinous meal.

4. Two of 30 mice were infected when fed moist feces from other mice appearing during the first 48 hours following the first meal of trichinous meat; whereas, 9 of 30 mice were infected when fed moist feces from other mice resulting from the second trichinous meal.

5. Four of 40 rats were infected when fed moist feces from other rats appearing during the first 40 hours following the first meal of trichinous meat, while 18 of 40 rats were infected when fed moist feces from other rats resulting from the second meal.

6. Larvae and cysts recovered from the feces of rats for 96 hours following the first meal of trichinous meat ranged from 0.0 to 5.6 percent of the total cysts fed as compared to 5.6 to 92.2 percent following the second meal.

7. From feces of 20 mice 2.0 to 11.0 percent of the total trichinae cysts fed were recovered as larvae following the first meal and 8.0 to 25.0 percent resulting from the second meal. No cysts were recovered.

8. The greatest number of larvae and cysts expelled after a meal of trichinous meat occurred during the first 24 hours following the meal.

9. Mice expelled, after the first meal, a greater percent of the total cysts fed than rats, while rats expelled the greater percent following the second meal.

10. Feces containing cysts that were kept moist and fed to trichina-free rats and mice produced more infections than similar feces allowed to dry.

11. Sheathed larvae and cysts in bits of undigested meat were found to be infective stages of trichinae occurring in the feces.

12. Free larvae of trichinae in feces were short-lived. They did not live beyond 2 hours when exposed to drying or 4 hours under moist conditions.

13. Trichinae larvae in cysts remained active up to 8 days when exposed to drying conditions and 27 days under moist conditions.

14. Moisture is considered an important factor in sustaining active trichinae in feces in an external environment.

15. Feces containing cysts should be considered a dangerous but a short-term source of infection for *Trichinella.*

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RESEARCH NOTE

EXPERIMENTAL INFESTATION OF A ROCKY MOUNTAIN BIGHORN LAMB WITH *MELOPHAGUS OVINUS* (DIPTERA: HIPPOBOSCIDAE).

The sheep ked, *Melophagus ovinus,* is found on the domestic sheep, *Ovis aries,* throughout most of its present distribution, but has only rarely been reported from species of wild sheep and has never been proven to breed on them. In North America, Ferris and Cole (1922, Parasitology **14:** 178–205) reported a new subspecies, *M. ovinus montanus,* from mountain sheep, presumably *Ovis dalli,* on the Alaska-Yukon boundary. Bequaert (1942, Ent. Am. **22:** 1–210) stated that the specimens from those mountain sheep appear to be the same race as those from domestic sheep, and suggested that the infestations were acquired from imported domestic sheep. There appear to be no reports of *M. ovinus* on the Rocky Mountain bighorn, *Ovis c. canadensis,* but few such sheep have been available for examination. Since bighorn rams have been known to associate with and breed with domestic ewes (Pulling, 1945, J. Wildl. Mgt. **9:** 82–83; Smith, 1954, State of Idaho, Dept. Fish and Game Wildl. Bull. No. 1: 154; Pillmore and Teague, 1955, Colo. Cons. **4:** 22–26), there is a possibility of introduction of the sheep ked into bighorn herds. Overlap of range might also allow indirect transfer of keds from domestic sheep to bighorns.

In the fall of 1959, the authors, working with the Montana Cooperative Wildlife Research Unit, were entrusted with a 4-month-old male bighorn lamb, *Ovis c. canadensis.* The lamb had been obtained when a week old from the Wildhorse Island herd, Flathead Lake, Montana, and reared by personnel of the Montana Fish and Game Department. It seemed to be an excellent opportunity to attempt experimental infestation of a bighorn with ectoparasites of domestic sheep. Consequently, on October 10, 1959, a number of *M. ovinus* and sheep biting lice, *Damalina ovis,* were collected from a farm flock of domestic sheep in the Bitterroot Valley near Florence, Ravalli County, Montana. Sixteen keds and about a dozen lice were placed on the right front shoulder of the lamb. The remaining insects were preserved for identification purposes. Lice were not seen again, probably because of the small number used and their poor condition due to handling. During the following 3 months, casual examinations revealed keds several times. On October 12 and 15 keds were observed on the right front shoulder and left hind leg, respectively; and from October 25 to November 8 periodic examinations revealed keds on the scrotum. On Feb. 3, 1960, almost 4 months after the initial exposure, a careful examination was made of the lamb, especially in the inguinal region. Seven male and two female *M. ovinus* were collected along with one pupal case containing a partially developed ked and two other pupal cases from which the keds had already emerged. Fragments of three additional pupal cases were seen but not collected. All of the keds appeared to have emerged relatively recently. One of the females was being inseminated when collected and the other appeared to be carrying a larva. On February 21, the lamb was again examined and nine additional keds were collected from the inguinal, rump, and neck regions. Five of these were reproducing females, one was a newly emerged female, and the other three were males. Seven pupae and five pupal cases were collected from both the neck and rump. These recoveries prove that the keds which were originally placed on the lamb had reproduced and indicate that their offspring were capable of reproduction.

The success of this experiment indicates that an infestation of *M. ovinus* could survive for long periods on an individual bighorn and be passed to other bighorns. Since the sheep ked is known to adversely influence the health of domestic sheep (Schwardt and Matthysse, 1948, Cornell Univ. Exp. Sta. Bull. 844), it might also have this effect on the bighorns. This would suggest that management of bighorns should include prevention or limitation of contact with domestic sheep.—CLYDE M. SENGER AND DONALD J. FORRESTER, *Montana State University, Missoula.*

DAUBAYLIA POTOMACA, A NEMATODE PARASITE OF *HELISOMA TRIVOLVIS*, TRANSMISSIBLE TO *AUSTRALORBIS GLABRATUS**

ELI CHERNIN, EDWARD H. MICHELSON AND DONALD L. AUGUSTINE

In a search for organisms which might serve as agents of biological control for the schistosome-bearing snail, *Australorbis glabratus,* the senior author examined aquatic snails collected during the summer of 1958 in the vicinity of the University of Michigan Biological Station, Cheboygan, Michigan. Dissection of the heart and other organs of *Helisoma trivolvis* disclosed nematodes subsequently identified through the courtesy of Mrs. M. B. Chitwood (1959) as *Daubaylia potomaca* Chitwood and Chitwood, 1934.

This report concerns preliminary observations on the distribution of infection with *D. potomaca* among *H. trivolvis,* on its transmission in the laboratory to *Australorbis glabratus,* and on the pathology produced by the nematode in these snails. Notes on the life history of the parasite are included.

MATERIALS AND METHODS

Snails were collected in 1958 from the following 4 areas near the Biological Station: (a) Sedge Point Pool, a beach pool on Douglas Lake, 1.5 miles north of the Station; (b) the shallow western edge of North Fishtail Bay on Douglas Lake, 1.7 miles northeast of the Station; (c) the west branch of the Maple River, 5 miles west of Pellston, Michigan; and (d) Fontinalis Run, a small stream at the head of Bourasau Bay on Burt Lake, about 3 miles southeast of the Station. These groups of snails were maintained at the Station on lettuce in separate battery jars containing well-water. In addition, during the summer of 1959, Dr. Kathleen L. Hussey kindly sent to Boston *H. trivolvis* collected from Sedge Pool, and from a fishing site on the Maple River 1.5 miles west of Pellston.

Since the nematodes were first encountered in the heart of *H. trivolvis,* and time did not allow exhaustive study of various organs from each snail, the following procedure was evolved for the examination of snails collected from the field in 1958. Each snail was measured, and the area over the heart cleared of shell. The pericardium was opened with a fine needle and the heart freed by cutting the base of the ventricle and the apex of the auricle with iris scissors. The intact heart was then placed in a few drops of a physiologically balanced "handling solution" (HS-unmodified; Chernin, 1957), in a 27 by 8 mm watch glass, and teased apart with fine needles under a dissecting microscope. Worms were separated from the tissues, counted, and fixed in hot (60 C) 70 percent alcohol containing 5 percent glycerine, in which they were preserved for further study; more rarely hot 5 percent formalin was used as a fixative. In many cases, the dissected heart, whether positive for worms or not, was then transferred to a slide in a small drop of handling solution, a No. 1 coverslip was added, and this "press" preparation was examined in toto under the compound microscope (100×). The combined procedures proved helpful since the nematode infection in any one snail might be manifested by the presence in the tissues of any or all of the stages from eggs and larvae to adult males and females. In a few instances the carcasses of heart-positive snails were macerated with scissors and subjected to digestion with pepsin-HCl. This disclosed that worms were sometimes present, frequently in large numbers, elsewhere in the body as well as in the heart.

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More detailed dissections were later undertaken on specimens of *H. trivolvis* from Fontinalis Run which had been air-shipped to Boston in mid-August, 1958. These snails were maintained in our laboratory at about 26 C in an aerated 3-gallon battery jar liberally supplied with lettuce and watercress. Selected specimens of *H. trivolvis* and *Australorbis glabratus* (strains PR-I and PR-II; see Chernin and Schork, 1959), suspected of being infected, were fixed in Zenker-acetic acid or Newcomer's solution, embedded in paraffin under vacuum, sectioned at 9.5 to 13 microns, and stained with haemalum and eosin (H-E) or haemotoxylin-azure II-eosin (H-AzII-E).

Specimens of the Michigan *D. potomaca* have been deposited in the U. S. National Museum (Helm. Coll. No. 56319), through the courtesy of Mrs. M. B. Chitwood.

Local Distribution of D. potomaca

The *H. trivolvis* collected in 1958 were adults ranging from 10 to 22 mm in diameter, with most of the specimens in the 14 to 20 mm range. Examination of hearts disclosed that infection was present among the snails from only one (Fontinalis Run) of the collecting sites (table I). Ten heart-positive snails, all *H. trivolvis* 16 to 21 mm in diameter, contained from 0 to 157 larvae, adult worms, or both, per heart; included among these 10 was 1 heart which was devoid of worms but contained 10 eggs, and 2 hearts which contained 5 and 12 eggs, respectively, in addition to larvae, adults, or both. In addition, the heart of a 9-mm specimen of *Helisoma campanulatum* from Fontinalis Run contained 7 adults and 2 larvae.

TABLE I. *Examinations of* Helisoma trivolvis *for infection with* Daubaylia potomaca. (*Collecting locales all in the vicinity of the University of Michigan Biological Station, Cheboygan, Michigan.*)

Collected		Dissected		
Place	Date	Dates	Number	Number positive
W. Branch Maple R.	8-11-58	8-11 to 8-16-58	64*	0
Fish. Site Maple R.	**	8-12-59	25	0
Sedge Pool	7-29-58	8-9 to 8-12-58	30	0
" "	8- 6-58	8-11 to 8-12-58	31	0
" "	**	8-12-59	15*	0
N. Fishtail Bay	8- 6-58	8-9 to 8-12-58	19	0
		Subtotal	184	0
Fontinalis Run	7-26-58	8-10 to 8-17-58	7	7
" "	8-13-58	8-13 to 8-15-58	12*	4*
		Subtotal	19	11

* Group includes one specimen of *Helisoma campanulatum*.
** Collected by Dr. Kathleen L. Hussey in July, 1959. Size range 10 to 14 mm.

The only other parasite encountered during this survey was *Megalodiscus temporatus*, rediae of which were found in the hearts (1 to 21 rediae per heart) of 31 of the 61 nematode-negative *H. trivolvis* examined from Sedge Pool in 1958. This infection was not observed in snails of the same species from the other collecting areas.

Transmission of D. potomaca *to* A. glabratus

On September 25, 1958, 23 laboratory-reared adult *A. glabratus* (strains PR-I and PR-II) were placed in a battery jar containing 3 live *H. trivolvis* and the carcasses of several others which had recently died (all shipped from Fontinalis Run). The last of the *Helisoma* died on October 20, at which time 2 (PR-II) of the surviving 17 *A. glabratus* were dissected and found heavily infected with *D. potomaca;* 2 days later, 2 additional *A. glabratus* (PR-I) were dissected and also found heavily

infected. On the latter date an additional 15 laboratory-reared *A. glabratus* (PR-I) were marked and placed in the jar with the survivors; on November 6 (15 days after exposure of this group), 1 of the marked snails was examined and found infected with *D. potomaca.*

Groups of marked *A. glabratus* of both strains were subsequently added to the same battery jar at 1 to 3 week intervals through the winter and spring. Water in this jar was not changed during these studies, although fresh water was added as needed to make up loss due to evaporation. Occasional examinations during this period disclosed the continued presence of the infection in successive groups of *A. glabratus,* although prevalence seemed to decline gradually for no apparent reason. A final batch of 50 *A. glabratus* introduced into the aquarium on April 15, 1959, was dissected between May 20 to 28 but only 1 of the 33 survivors was found infected. *A. glabratus* which had survived from earlier exposure groups were also dissected at this point, but only 1 of 32 proved to be positive.

Mortality among introduced lots of *A. glabratus* varied widely, but on occasion exceeded 75 percent after 3 to 4 weeks. Since adequate controls and means of assessing frequency of infection were lacking, it is not certain whether all of these deaths were actually due to infection with *D. potomaca.* Before and during the period of these studies several thousand *A. glabratus* from our stock colonies were dissected or sectioned in connection with other work, but in no instance was infection with *D. potomaca* observed.

Pathological and Life History Studies

All or portions of 4 naturally-infected *H. trivolvis* and 9 laboratory-infected *A. glabratus* were sectioned and examined. The following applies to both species of snails, except as noted, and data from freshly dissected specimens are also included.

Chitwood and Chitwood (1934) reported finding *D. potomaca,* presumably adults, only in the "pulmonary cavity" of *H. trivolvis.* In the present study, however, adult males and females, larvae (apparently of all stages), and eggs (in all stages of development from 1 cell to fully embryonated), were found in nearly all tissues and blood spaces. Parasites were rare in nervous tissue and were not seen at all in: (a) the buccal cartilages (radular carrier); (b) in the glandular structures associated with the female genital tract (e.g., the nidamental gland, the uterus, the albumen gland, and the seminal receptacle); or (c) in the prostatic gland of the male genital system. However, the connective tissue surrounding these organs contained parasites. The heaviest concentrations of worms, eggs, or both, were observed in the heart (figs. 1 to 4), the saccular portion of the kidney, the rectal ridge (fig. 11), the musculature of the foot and gizzard (figs. 5, 8, 9), the blood sinuses of the mantle (figs. 6, 7), and the blood sinus over the columellar muscle. The common, but not exclusive, occurrence of the worms in the heart and blood sinuses suggests that they may migrate throughout the animal via the circulatory system, although their presence in such structures as the wall of the gizzard indicates that they may traverse dense tissues as well.

Recognizable pathology produced by worms or eggs was frequently lacking. When present, pathologic changes appeared to be largely limited to local destruction of muscle and connective tissue fibers (figs. 5, 7, 11). In such areas, free nuclei

and varying degrees of amoebocytic infiltration were occasionally seen. In one infected *A. glabratus,* a fibrous granuloma was found surrounding several eggs and an adult worm, all of which, judging from their staining reactions, were dead (fig. 6). Viable eggs were found in nearly all tissues (figs. 8, 9, 10, 12), in proximity to adult worms, or isolated, but no consistent cellular reaction to them was observed.

D. potomaca freshly teased from the heart or other tissues are colorless, active worms which tend to migrate back into bits of tissue or mucous, or to become inextricably tangled with each other if left undisturbed in handling solution at room temperature. Cleaned of adhering tissue and transferred to small watch glasses of chilled handling solution, adult worms survived 3 to 6 days at 5 C, and resumed active movements within a few minutes after the dishes were removed from the refrigerator. Adult males and females of *D. potomaca* are nearly equal in size (less than 2 mm in length), and in both the tail is recurved. Detailed morphological features of *D. potomaca* are given in the original description of the nematode by Chitwood and Chitwood (1934).

The uterus of mature females usually contained a single undeveloped egg. As indicated earlier, eggs found in pressed tissues of any one snail host ranged from the completely undeveloped to the fully embryonated state containing an active larva. A small number of eggs in heart tissue press preparations measured 64 to 71 microns long (mean 66 microns) and 25 to 32 microns wide (mean 28 microns). These findings have been generally corroborated by tissue sections, although not all snails infected with the nematode have also been found to contain eggs. The eggs are characteristically oval in outline and usually lie embedded in tissue rather than in free spaces. However, a few objects, thought to be eggs of *D. potomaca,* were seen in the intestinal lumen of one infected *A. glabratus.* In still another heavily infected *A. glabratus,* a single nematode resembling *D. potomaca* was found in sections of the snail's gut (fig. 13), and on a few occasions eggs were found in the snail feces.

Adult worms freshly recovered from laboratory-infected *A. glabratus* and placed in small dishes of charcoal-treated tap water (at 26 C), died within 24 hours, and eggs in utero disintegrated by the fourth day. When handling solution was substituted for tap water, adult worms in one experiment survived for 4 days and some development of eggs in utero was observed. In a second experiment using handling solution, several eggs were deposited by the females on day 1, underwent development, and yielded 6 motile, long-tailed, rhabditiform larvae on day 4, 3 more on day 5, and another on day 6; by day 7 all adults and larvae had died despite the addition to the fluid, on day 4, of a small amount of dispersed *A. glabratus* feces.

DISCUSSION

It is not now possible to assess the significance of *D. potomaca* in terms of capacity to produce mortality or morbidity among snails. Our limited data suggest that the parasite may be effective in killing *A. glabratus* under some conditions, as yet undefined. Worm burden and individual variations in response among infected snails are probably important in determining snail survival. We have observed seemingly unaffected *A. glabratus* or *H. trivolvis* which, upon dissection, contained

hundreds of worms; conversely, sluggish or moribund snails have been known to yield only a few of the nematodes.

The only snails found in Fontinalis Run besides *H. trivolvis* were numerous ancylids and rare specimens of *Helisoma campanulatum, Bulimnaea* sp. and *Campeloma* sp. Except for *H. campanulatum* (one specimen of which was infected), time did not permit examination of the other snails for *D. potomaca.* During July and August 1959, 145 *Helisoma caribaeum* from 4 localities in Puerto Rico, and 35 from 2 localities in St. Croix, V. I., were examined for *D. potomaca* (by E. H. M.), as were 35 *H. trivolvis* of an albino strain from a stream and pond in Wellesley, Mass. (by E. C.), but no infections were encountered in either series. Thus, the currently known natural hosts of *D. potomaca* are *Helisoma trivolvis* and *H. campanulatum* in northern Michigan (present study), and *H. trivolvis* in the Potomac River (Chitwood and Chitwood, 1934). However, the successful transmission of *D. potomaca* to *A. glabratus* suggests that related planorbid snails, and possibly others, may be found infected in nature or be potentially susceptible to infection under laboratory conditions.

According to Chitwood and Chitwood (1934), other species of *Daubaylia* include *D. seistenensis* (*Cephalobus seistenensis* Baylis and Daubney, 1922) from *Gyraulus convexiusculus* collected in Persia, and ". . . *Daubaylia* sp. (larvae) from *Philomycus carolinensis* (muscles)." More recently, a new species of *Daubaylia, D. dewiti,* has been described from *Planorbis contortus* collected in Holland (Schuurmans Stekhoven, 1956); although no details are given, this nematode, like the Michigan *D. potomaca,* has apparently been found in the deep tissues of the snail. It is of interest that except for the reference to larvae of *Daubaylia* sp. from the slug, *Philomycus carolinensis,* all other infections with species of *Daubaylia* have been reported from snails of the Family Planorbidae. A general review of the nematodes of gastropods appears in a paper by Mengert (1953).

Chitwood and Chitwood (1937) separated the nematodes found in snails into 6 groups as follows: "(1) nematodes normally free-living (living upon decaying plant and animal matter) and plant parasites which may pass through the digestive tract uninjured; (2) nematodes living as obligatory parasites in the digestive tract; (3) nematodes living as parasitic larvae in the foot muscle and having a free-living adult stage; (4) adult nematodes living in the genital organs; (5) agamic nematodes and Nematomorpha which live in the body cavity and leave the host upon reaching maturity, subsequently passing the remainder of their life as free-living forms; and (6) nematode parasites of vertebrates, the larval stages of which occur in snails." Although the life cycle of *D. potomaca* in *H. trivolvis* or *A. glabratus* is not known, it would not seem to fit into any of the foregoing categories since eggs, larvae, and adults of the parasite may be present simultaneously within and throughout one snail host. It is possible that these findings merely reflect rapidly successive superinfections, though this seems rather improbable. Under these circumstances, several life history possibilities suggest themselves, viz: (a) eggs may hatch internally resulting in auto-infection; (b) eggs (or larvae?) may work their way through the tissues of the living host and escape via the epithelium, the kidney, the reproductive tract, or the gut, and give rise to an infective stage externally with or without extrinsic multiplication; (c) transmission among snails may occur by their exposure to, or ingestion of, parasite stages from a recently dead or from a disintegrated host.

Further experimental work seems desirable to determine the nature of the life cycle, to develop methods for detecting infection short of sacrificing the host, to provide suitable means (perhaps cultivation in vitro) for maintaining stocks of the parasite in the laboratory, and to ascertain the possible usefulness of *Daubaylia potomaca* in the biological control of snails of medical or veterinary importance.

SUMMARY

Daubaylia potomaca Chitwood and Chitwood, 1934, originally described as a parasite of *Helisoma trivolvis* in the Potomac River, is now reported as a natural infection in *H. trivolvis* and *H. campanulatum* from a stream in the vicinity of the University of Michigan Biological Station, Cheboygan, Michigan. Transmission of *D. potomaca* from *H. trivolvis* to *Australorbis glabratus* was accomplished in the laboratory, and observations indicate that the life cycle may be completed in 15 days or less. Eggs, larvae and adult nematodes of both sexes were found simultaneously in deep tissues. The known geographic distribution of the parasite and the pathology produced by it are described, and its possible use as a means of biological control for *A. glabratus* is noted.

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Explanation of Plates I and II

Daubaylia potomaca in *Australorbis glabratus* and *Helisoma trivolvis*

Figure 1. Gross specimen of heart, slightly teased apart and fixed with formalin, heavily infected with nematodes. Worms appear as thin white filaments within and along the margins of the heart mass (arrows). *H. trivolvis,* natural infection. (Unstained; approx. × 17).

Figure 2. Section of heart with nematodes in lumen and tissues of ventricle. Heart was removed, prior to fixation, from a juvenile *H. trivolvis* which had developed in the experimental aquarium and became infected. Exposure period not known. (10.5 microns; H-E; × 285).

Figure 3. Concentration of worms and eggs in longitudinal section of entire heart. Organ was removed, prior to fixation, from an *A. glabratus* (PR-II) 25 days after exposure to infection. (9.5 microns; H-E; × 143).

Figure 4. Section of heart in situ. Massive invasion of tissues and chambers with various stages of the parasite. *A. glabratus* (PR-I) 27 days after exposure to infection. (12.5 microns; H-AzII-E; × 143).

Figure 5. Nematodes in section of foot musculature. *A. glabratus* (PR-I) 27 days after exposure to infection. (12.5 microns; H-AzII-E; × 684).

Figure 6. Section of mantle collar. Granuloma containing encapsulated eggs and nematodes. *A. glabratus* (PR-I) 27 days after exposure to infection. (12.5 microns; H-E; × 684).

Figure 7. Nematodes in blood space in section of mantle collar. *H. trivolvis.* Natural infection. (12.5 microns; H-E; × 285).

Figure 8. Section of dense musculature of gizzard wall. A nematode, an undeveloped egg (arrow), and an adjacent developed egg are visible. *A. glabratus* (PR-II) 25 days after exposure to infection. (10.5 microns; H-E; × 413).

Figure 9. Section of gizzard near lumen (lower left). An embryonated egg is seen in longtitudinal section and an undeveloped egg in cross section. Faint lines between the eggs (arrow) are egg shells. *A. glabratus* (PR-I) 27 days after exposure to infection. (10.5 microns; H-E; × 684).

Figure 10. Developing egg of *D. potomaca* in connective tissue of ovotestis. *A. glabratus* (PR-I) 27 days after exposure to infection. (10.5 microns; H-AzII-E; × 684).

Figure 11. Section of rectal ridge with nematodes in destroyed area. In addition, several eggs lie in the dense tissue along the right margin of the ridge, and a granuloma appeared to be forming around 2 of the eggs at the apex of the ridge (arrow). *A. glabratus* (PR-I) 27 days after exposure to infection. (10.5 microns; H-E; × 143).

Figure 12. Undeveloped egg of *D. potomaca* in section of wall of hind gut (gut lumen on the right). *A. glabratus* (PR-I) 27 days after exposure to infection. (10.5 microns; H-E; × 498).

Figure 13. Section of hind gut with cross section of nematode in lumen. Other organs heavily parasitized with *D. potomaca. A. glabratus* (PR-I) 27 days after exposure to infection. (10.5 microns; H-E; × 684).

PLATE I

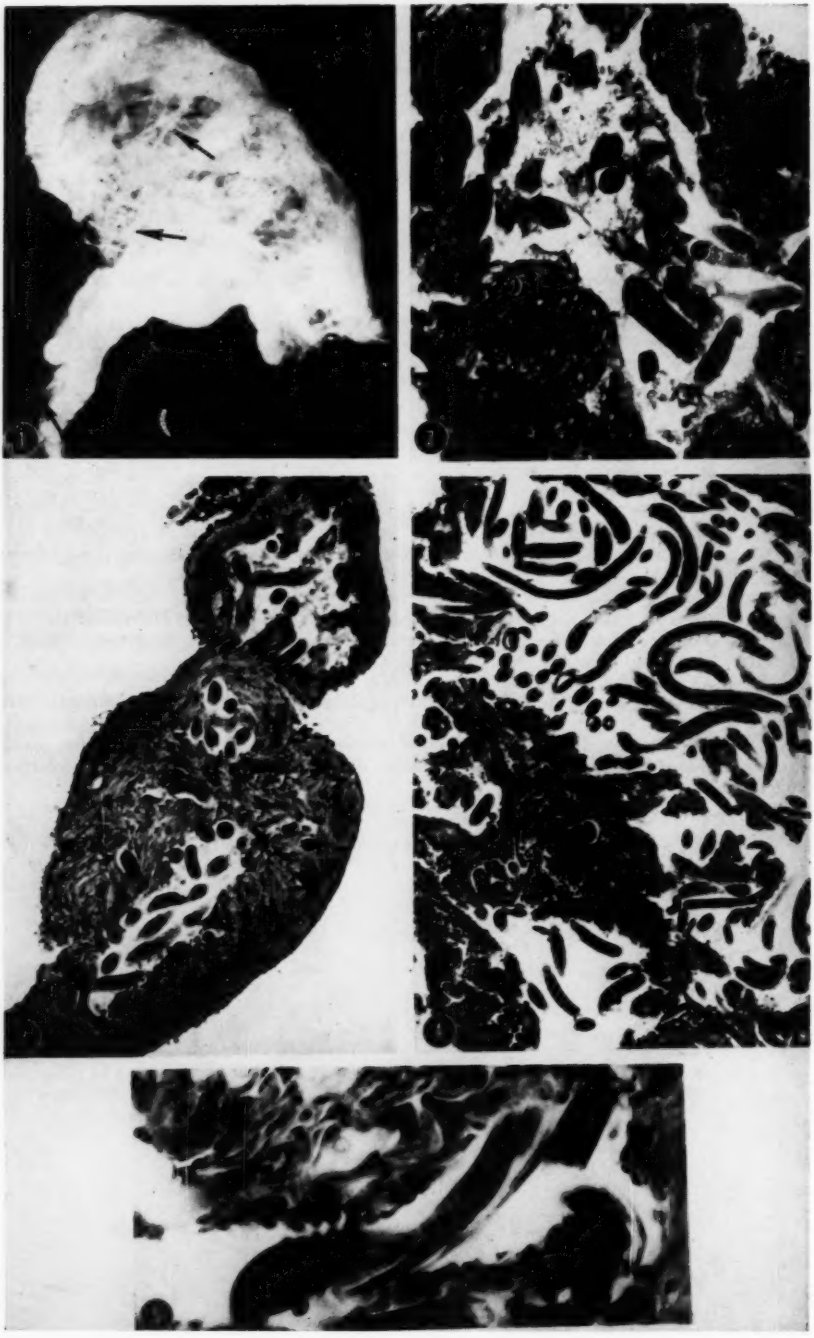
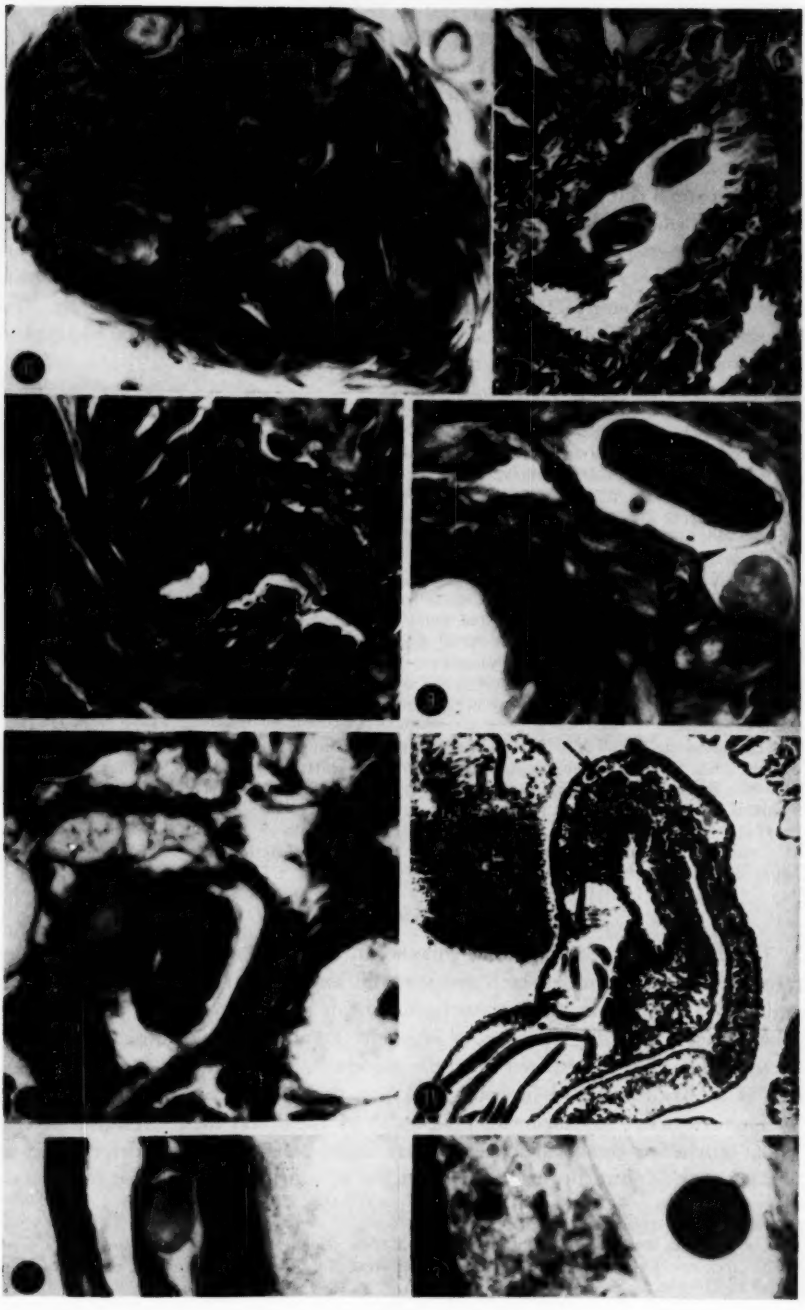


PLATE II



THE RHOMBOGEN PHASE OF *DICYEMA SULLIVANI* McCONNAUGHEY (MESOZOA: DICYEMIDAE)

BAYARD H. McCONNAUGHEY

Dicyema sullivani McConnaughey, 1949, was described from nematogens only, found in a small octopus of the *Octopus bimaculatus* complex near Rosarita Beach, Lower California. Subsequent to the publication of this description (McConnaughey, 1949) the *Octopus bimaculatus* complex was divided into 2 species (Pickford and McConnaughey, 1949), the octopus in which *D. sullivani* had been taken being assigned to a new entity *Octopus bimaculoides* Pickford and McConnaughey. The present note will complete the description of the known portions of the life cycle of the mesozoan and extend its geographical range.

Rhombogen phase: Adult rhombogens mostly 0.7 to 1.5 mm. long, commonly more robust appearing than nematogens; somatic nuclei of trunk cells mostly divided amitotically, often unequally into 2 to 4 or more smaller nuclei, making the number and size of nuclei in the somatic cells very irregular; protein reserves in these cells lightly staining spheroid rather irregular granules, many showing 1 or 2 dark staining dots (?mitochondria) on them (fig. 4); axial cell ending broadly at the base of the metapolar cells (figs. 1 and 2).

Infusorigens (figs. 4 and 6) small but numerous, 10 to 12 commonly present, their axial cells no larger than the well developed peripheral egg cells, the entire infusorigen smaller than an infusoriform larva; very few male germ cells present, each infusorigen commonly containing only 1 or 2 spermatogonial cells and 2 to 4 cells in various stages of spermatogenesis at any one time; paranuclei and sometimes the axial nucleus of rhombogen may divide amitotically, often unequally, resulting in the presence of more free nuclei in the axial cell of the rhombogen than expected from the number of infusorigens plus one.

Near each infusorigen in some specimens are found one or more degenerating cells showing basophilic cytoplasm and enlarged pycnotic chromosomes. These are probably detached unfertilized egg cells.

Infusoriform (figs. 9 and 10) rather large, about 40 to 48 microns long, tapering to a bluntly rounded point behind, of typical structure and form, with large refringent bodies and each of the 4 cells in the urn containing 2 free nuclei and 1 germ cell; usually only 2 or 3 mature infusoriforms associated with each infusorigen at any one time.

Host: Octopus bimaculoides Pickford and McConnaughey.

Localities: Near Rosarita Beach, Lower California; Mission Bay, California; La Jolla, California; Point Loma, California.

Discussion

The most salient features of the rhombogen phase of this species are the marked irregularities in the nuclei, both somatic and axial, and the small size but large number of infusorigens. Spermatogenesis in this species appears to be too limited to keep pace with the production of egg cells, resulting in the presence of one or more degenerating unfertilized egg cells associated with many of the infusorigens. This is especially marked in young primary rhombogens (figs. 5 and 6). The development of the infusoriforms appears to be rapid and their liberation prompt, so that only a few embryological stages are found between the infusorigens and the fully developed infusoriforms, and only a few infusoriforms are found in the axial

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cell of the rhombogen associated with each infusorigen. The number of infusorigens is so great however that the axial cell of the rhombogen appears about as full of infusorigens as is the case in species with larger more productive infusorigens.

This species is unusual among sexual animals in that sperm production appears to be more limited than that of egg cells.

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Explanation of Plate I

(All figures drawn with the aid of a camera lucida)

Figure 1. Calotte and anterior part of trunk of rhombogen showing irregularity and amitotic division of somatic nuclei.

Figure 2. Calotte of another rhombogen. Note in both 1 and 2 the characteristic broad blunt ending of the axial cell at the base of the metapolar cells.

Figure 3. Entire rhombogen to show general proportions and content of axial cell.

Figure 4. Young primary rhombogen from a small (10 g) octopus. Its axial cell contains 10 infusorigens, each with 1 to 4 paranuclei, some of them in process of amitotic division, and from 1 to 4 basophilic pycnotic degenerating egg cells. That this is a young rhombogen, rather than a spent one, which has discharged its larvae and is exhibiting extensive degenerative phenomena, is indicated by its slenderness (cf. fig. 3, fully active rhombogen), by the fact that no mature sperm or any stages of development of infusoriforms are present, as is usually the case in spent rhombogens, and by the fact that this preparation was made from a small octopus in which early rather than spent rhombogen would be expected.

Figure 5. An infusorigen from the rhombogen shown in figure 3 showing the free nucleus, 1 spermatogonium and 1 primary spermatocyte in its central cell, plus 4 fully formed sperm cells. Developing egg cells are on its periphery, the largest with a sperm attached to its edge. The paranucleus lies nearby in the axial cell of the parent rhombogen. The trunk cells of the rhombogen show the typical protein reserve granules.

Figure 6. One infusorigen (the fourth from the anterior end) from the rhombogen shown in figure 4, showing a degenerating cell and 3 paranuclear fragments nearby.

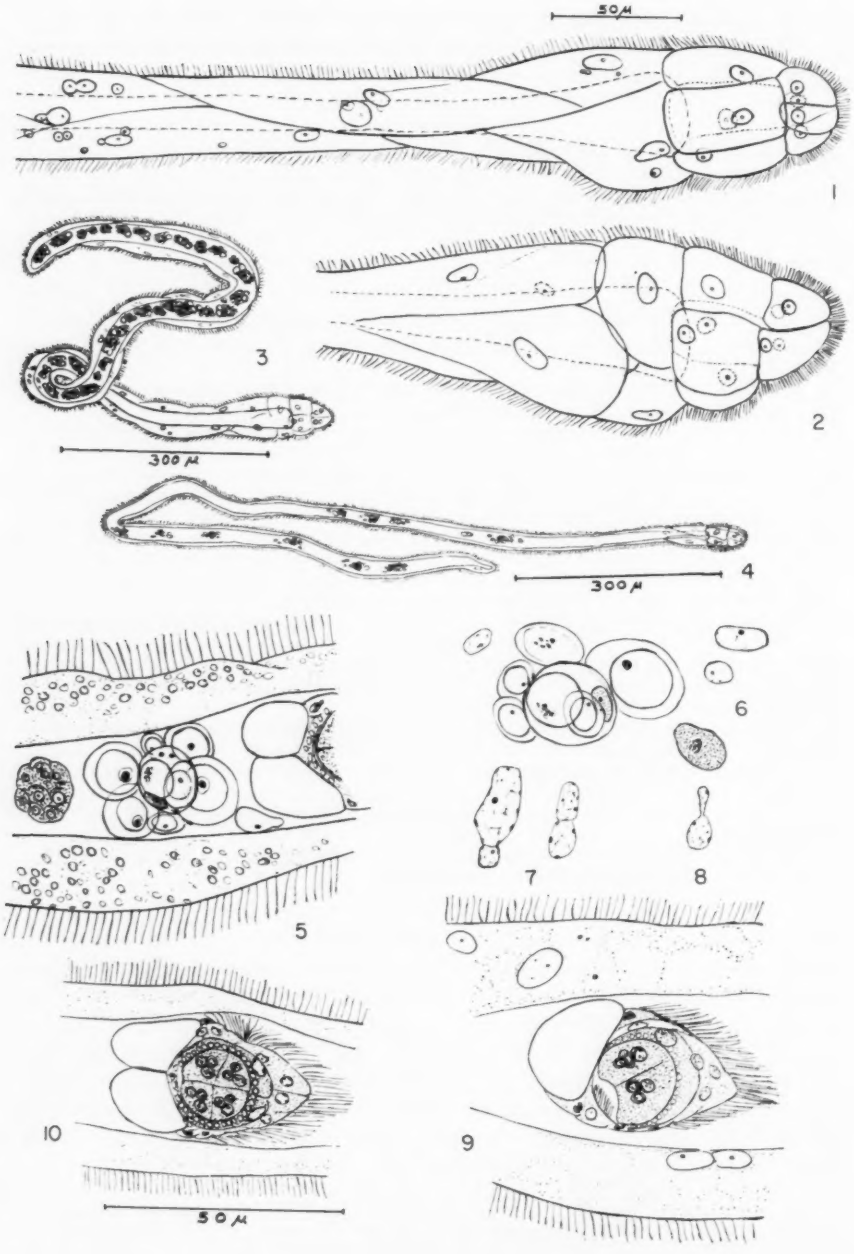
Figure 7. Paranuclei associated with infusorigens 9 and 10 respectively from the same rhombogen, in course of amitotic division.

Figure 8. One of the somatic nuclear fragments from the same rhombogen in course of amitotic division.

Figure 9. Mature infusoriform larva, parasagittal optical section.

Figure 10. Infusoriform, frontal optical section.

PLATE I



ACANTHOCEPHALA FROM SHORE BIRDS OF EGYPT, WITH THE DESCRIPTION OF A NEW SPECIES OF *MEDIORHYNCHUS*

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A number of specimens of the acanthocephalan genera *Centrorhynchus* and *Mediorhynchus* have been collected from the intestines of shore birds of Egypt by the United States Naval Medical Research Unit No. 3. The specimens have been forwarded to the writer by Dr. Robert E. Kuntz. One group of worms of the genus *Mediorhynchus* differs from previously described species; these are described as a new species, *Mediorhynchus kuntzi*. The following is a list of the Acanthocephala collected from shore birds with the hosts and localities of each.

Centrorhynchus lancea (Westrumb, 1821)

Six specimens, PE1736A, were collected from *Burhinus senegalensis* (stone curlew) in Wadi Nassim, Isna Qena Province.

Four immature specimens, PE1118A, were collected from *Burhinus oedicnemus saharae* (stone curlew) in Giza Province.

One immature specimen, PE723A, was collected from *Hoplopterus spinosus* (plover) in Qalyoubiya, Qena Province.

Meyer (1932) has reported this parasite from *Vanellus vanellus, Charadrius apricarius, C. alexandrinus, Philomachus pugnax, Himantopus himantopus, Eudromias morinelli, Oedicnemus oedicnemus,* and *Cuculus canorus.* These are all from Europe. The occurrence of this parasite in Egypt represents a new geographical locality, and *Burhinus senegalensis* and *Hoplopterus spinosus* are new host records. *Oedicnemus oedicnemus* is a synonym for *Burhinus oedicnemus saharae.*

Centrorhynchus milvus Ward, 1956

Eight specimens, PE1678A, were collected from *Burhinus senegalensis* (stone curlew) in Wadi Nassim, Isna Qena Province. These specimens are immature, about 5 mm in length.

Sixteen immature specimens, PE1118A, were collected from *Burhinus oedicnemus saharae* (stone curlew) in Giza Province.

One immature specimen, PE3464A, was collected from *Hoplopterus spinosus* (plover) at Lake Qarun, Faiyum.

Adults of this species were described from *Milvus migrans* (kite) in Egypt (Ward, 1956). Golvan (1956) reported this species from *Milvus migrans* in Senegal, and he also found juveniles encysted in *Boedon fuliginosus* (Colubridae). The birds, *Burhinus senegalensis, Burhinus oedicnemus sarahae,* and *Hoplopterus spinosus* represent new host records for this species.

Centrorhynchus corvi Fukui, 1929

One immature specimen, PE3464A, was collected from *Hoplopterus spinosus* (plover) at Lake Qarun, Faiyum.

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This species was described from *Corvus* sp. by Fukui (1929) in Japan. Yamaguti (1939) has reported it from *Corvus corone interpositas* and *Pica pica sericea* in Japan. The occurrence of this parasite in Egypt represents a new geographical locality, and *Hoplopterus spinosus* is a new host record.

Mediorhynchus kuntzi n. sp.
(Figs. 1–3)

Description: With the characteristics of the genus *Mediorhynchus*. Body cylindrical, slightly swollen in anterior region. Pseudosegmentation present except in anterior swollen region. Females 16 to 20 mm by 1 mm in maximum diameter. Males (fig. 1) 15 to 18 mm by 0.7 mm in maximum diameter. Proboscis (fig. 2) in the form of a truncated cone, 0.6 to 0.7 mm by 0.4 mm at base. Large hooks (fig. 3) on anterior region of proboscis arranged in 12 to 14 spiral rows of 6 to 8 each. Spines on posterior region of proboscis in 20 to 22 spiral rows of 9 or 10 each. Characteristic shape of roots of large hooks shown in figure 3. Length of large hooks 0.04 to 0.06 mm. Small spines about 0.02 mm in length. Proboscis sheath 0.7 to 0.9 mm by 0.20 to 0.25 mm. Lemnisci slightly unequal in length, 2.2 to 3.1 mm by 0.15 to 0.19 mm. Testes, 1 to 1.2 mm by 0.25 mm, located in posterior region of body. Cement receptacle 0.5 by 0.2 mm. The females are immature and do not contain ensheathed embryos.

Type host: *Burhinus senegalensis* (stone curlew).

Locality: Wadi Nassim, Isna Qena Province, Egypt.

Type material: Holotype male and allotype female in U. S. N. M. Helm. Coll. No. 39043. Paratypes representing both sexes in Parasitology Collection, Department of Zoology and Entomology, University of Tennessee.

The new species resembles *Mediorhynchus tenuis* Meyer, 1931, which was described from *Saxicola bimaculata* and *Monticola solitarius* (Turdidae) in Egypt (Meyer, 1932). However, *M. kuntzi* differs from *M. tenuis* in body size and other respects. The males of *M. kuntzi* are up to 18 mm in length; *M. tenuis* males are 12 mm in length. The females of *M. kuntzi* are 16 to 20 mm in length, while the females of *M. tenuis* are up to 33 mm in length. Since the females of the new species are immature, it is probable that mature specimens would reach a greater length. The body of *M. kuntzi* shows a distinct pseudosegmentation, while pseudosegmentation is lacking in *M. tenuis*. The lemnisci of *M. tenuis* are relatively much longer than those of *M. kuntzi*. The cement glands of the new species are not arranged in pairs, as they are in *M. tenuis*.

SUMMARY

Acanthocephala are reported from shore birds of Egypt, as follows: *Centrorhynchus lancea, C. milvus,* and *Mediorhynchus kuntzi* n. sp. from *Burhinus senegalensis; Centrorhynchus lancea* and *C. milvus* from *Burhinus oedicnemus saharae; Centrorhynchus lancea, C. milvus,* and *C. corvi* from *Hoplopterus spinosus.*

A description of *Mediorhynchus kuntzi* n. sp. is given.

Burhinus senegalensis is a new host record for *C. lancea* and *C. milvus. Burhinus oedicnemus saharae* is a new host record for *C. milvus. Hoplopterus spinosus* is a new host record for *C. lancea, C. milvus,* and *C. corvi.*

Egypt is a new locality record for *C. lancea* and *C. corvi.*

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EXPLANATION OF PLATE I

All figures are of *Mediorhynchus kuntzi* n. sp. Drawings made with the aid of microprojection from stained whole mounts.

FIGURE 1. Adult male.
FIGURE 2. Proboscis.
FIGURE 3. Large hook from anterior part of proboscis.

RESEARCH NOTE

METACERCARIA OF *TRANSVERSOTREMA PATIALENSE* IN THE FRESH-WATER FISH *MACROPODUS CUPANUS*.

In a previous communication (Crusz, 1956, J. Parasit. **42**: 245) some suggestive data were presented in regard to *Cercaria patialensis* Soparkar infecting 4 percent of snails of the species *Melanoides tuberculata* in rice-fields at Godapola, Ceylon. Since then, this cercaria has been found by one of us (H. C.) to infect the same snail host in the Kirillapone Canal, Colombo (20 percent infection) and in rice-fields at Batalagoda, Ibbagamuwa (40 percent infection).

The rice-fields at Batalagoda are irrigated by fresh-water channels from the Batalagoda Wewa (tank or reservoir), which receives most of its water from the Deduru Oya river that has been dammed for this purpose. On the view that the final host of *C. patialensis* is most probably a fresh-water fish, the following fish were examined from time to time but found to be uninfected: *Rasbora daniconius, Esomus danrica thermoicos, Danio aequipinnatus, Puntius filamentosus, P. bimaculatus, Labeo porcellus lankae, Neomacheilus botia, Macrones vittatus,* and *Ophiocephalus punctatus.*

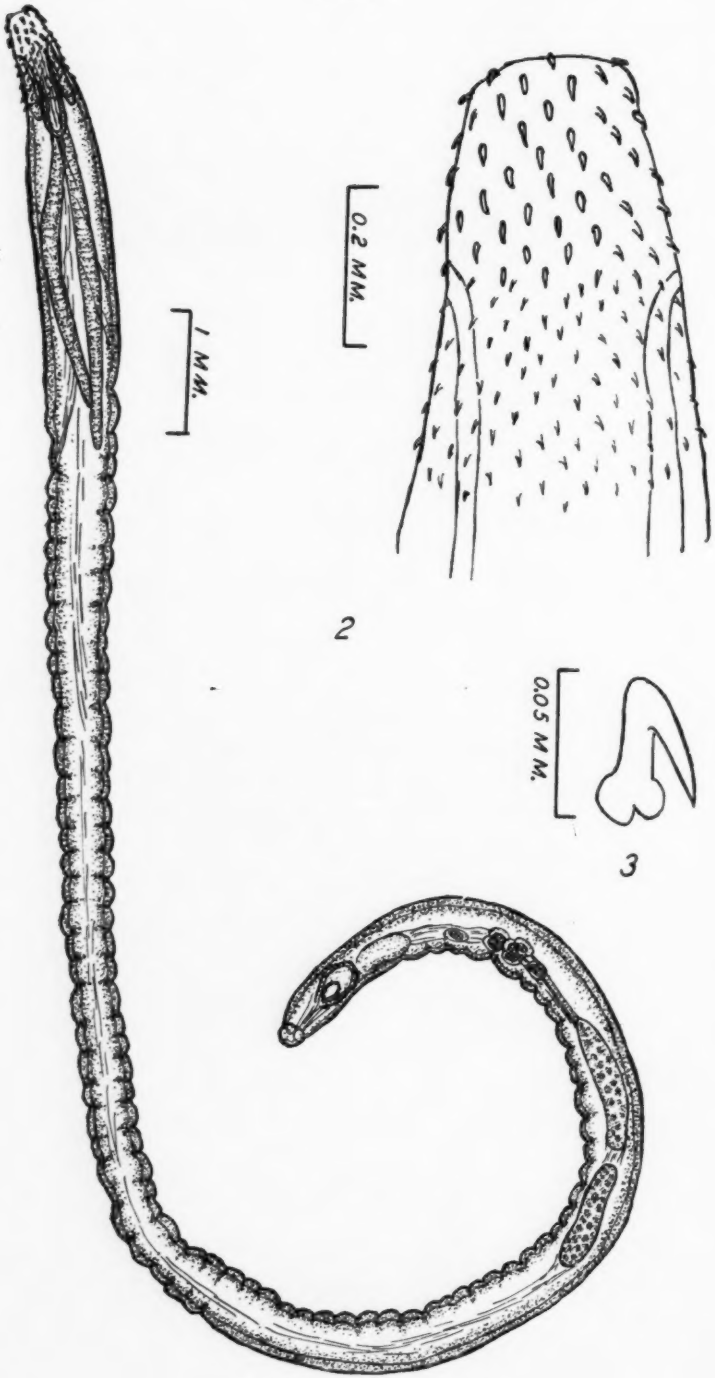
There was no way of limiting our investigation to a particular species or group of fish, since Witenberg (1944, J. Parasit. **30**: 179), who was the first to describe an adult *Transversotrema,* was not in a position to name its marine fish-host. A recent paper, however, by Velasquez (1958, J. Parasit. **44**: 449) helped us considerably. Velasquez found the metacercaria of *Transversotrema laruei* in the giant perch, *Lates calcarifer* (Bloch), from brackish waters of the Philippines. We therefore turned our attention to this fish, which is also found in Ceylon. But it is not to be seen at all in the irrigation channels at Batalagoda or in the Batalagoda Wewa, probably because of the damming of the river.

We, therefore, began looking for this trematode in other Perciform fishes of the irrigation channels at Batalagoda. Our efforts have been successful at the outset. The very first specimen of *Macropodus cupanus* (Cuvier), the palmyrah-fibre fish, examined on November 30, 1959, yielded two fine metacercariae of *Transversotrema patialense.* Unfortunately, their exact location inside the body of the fish could not be determined owing to the small size of both metacercariae and fish and also because microscopic examination was done after the viscera had been dissected out and teased in saline. Three other specimens of *M. cupanus* were also examined, but were uninfected.

The two metacercariae are undoubtedly those of *Cercaria patialensis.* Each of them is tailless and has a slight indentation at the mid-posterior end where the tail was once attached. Spermatozoa are abundant in the male genital ducts, but there are no eggs in the uterus. One specimen (0.7 mm in width) shows distinct follicular vitelline glands. In all these respects the metacercaria is much like that of *Transversotrema laruei* Velasquez, 1958, but larger. The following measurements, in millimeters, were obtained for the two specimens: length 0.323, 0.306; width 0.612, 0.700; diameter of pharynx 0.058, 0.048; diameter of acetabulum 0.102, 0.102.

There can be no doubt that *Cercaria patialensis* Soparkar, 1924, is the progenetic larval form of a species of *Transversotrema* Witenberg, 1944.—H. CRUSZ AND A. H. SATHANANTHAN, *Department of Zoology, University of Ceylon, Colombo, Ceylon.*

PLATE I



EFFECTS OF STARVATION AND LACK OF BILE UPON GROWTH, EGG PRODUCTION AND EGG VIABILITY IN ESTABLISHED RAT TAPEWORMS, *HYMENOLEPIS DIMINUTA**

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Lack of bile exerts profound effects upon establishment and persistence of the rat tapeworm, *Hymenolepis diminuta*, from ingested cysticercoids or after surgical transfaunation of adult worms (Goodchild, 1958). The nature of this influence is as yet unknown, but the effects are so striking and reproducible that further study of the phenomenon in rats seemed to be desirable.

Accordingly, in the following study, gross effects on tapeworms, egg production, and egg viability were investigated using single-worm infections in normal Wistar rats, in rats with biliary by-pass to the caecum, and in rats during starvation.

MATERIALS AND METHODS

One cysticercoid of *H. diminuta* was fed by stomach tube to each of 69 weighed Wistar rats (270 to 280 g) within 2 days of the same age. Mature cysticercoids, 18 days old, were obtained by dissecting infected flour beetles, *Tribolium confusum*, in saline.

On the 20th day after infection, the presence or absence of tapeworm eggs was checked by blending a small quantity of feces with approximately 100 ml of water in a Waring Blendor. The mixture was placed in two 50-ml tubes and centrifuged for 2 minutes at 1500 rpm. The supernatant water was discarded and approximately 15 ml of fresh water added to each tube. Salt was added in excess, and the mixture was poured back and forth between the two tubes; the mixture was concentrated in one tube, which was then centrifuged for 2 minutes at 1500 rpm. Approximately 10 ml of the surface material were removed to two 15-ml centrifuge tubes, water was carefully layered over the saturated salt mixture, and the tubes were centrifuged for 2 minutes at 3000 rpm. The eggs, when present, could be seen clearly as a golden layer between the salt solution below and the water above.

On the 20th day after infection: (1) 16 infected rats were taken off food and starved for the next 7 days; (2) an operation was performed on each of 25 infected rats to cannulate the bile duct to the caecum, according to the method given in a previous paper (Goodchild, 1958), and the rats were given food for the next 7 days, and (3) 15 unoperated infected rats remained on a normal diet for 7 days as a control series.

Feces were collected from each of 31 rats (9 starved, 15 bileless, and 7 normal) over the 7 days (days 21 through 27, post-infection). Feces were collected daily by placing an aluminum-foil tray, containing one or more paper towels to absorb urine, under each cage. Each day all of the feces were removed, wrapped in aluminum foil with a tag bearing the rat's number and the date of collection, and placed in the refrigerator until egg counts could be made.

Egg counts were performed as follows: Feces were removed from the refrigerator and weighed. The feces were placed in a Waring Blendor, approximately 150 ml of water added, and the contents blended for 2 minutes. The blended mixture was transferred to a 1000-ml erlenmeyer flask if the feces weighed more than 2 grams, and water was added to bring the mixture to 500 ml; if the feces weighed less than 2 grams, the mixture was placed in a 250-ml erlenmeyer flask and water was added to bring the mixture to 125 ml. The flask was placed on a magnetic stirrer and allowed to mix for approximately 10 minutes. While the mixture was stirring, two 6 ml samples were removed and placed in graduated centrifuge tubes. Salt was added in excess, the tubes were well shaken, water was carefully layered over the saturated salt mixture, and the tubes centrifuged for 2 minutes at 3000 rpm.

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* This investigation was supported (in part) by a research grant (PHS–E795) from the Institute of Allergy and Infectious Diseases, of the National Institutes of Health, Public Health Service.

All eggs were concentrated in the interface and all were removed from one of the tubes (or from both if eggs were scanty) by means of a micropipette. The eggs were diluted to a definite volume in a graduated tube and thoroughly shaken. Five 0.1-ml samples were removed and placed in depression slides and the eggs counted with the aid of a dissecting microscope. The five counts were used to determine the total number of eggs in the whole day's collection of feces.

From each day's collection of feces, 2 samples were selected at random from each series. From each sample, all the eggs from 100 ml of the blended mixture prepared for egg counts were concentrated, removed from each tube, and washed through two changes of water. The eggs in a small quantity of water were then mixed with powdered oatmeal, spread on small squares of filter paper, and placed in a petri dish with 10 beetles (*Tribolium confusum*) which had previously been starved for 2 days. The egg-oatmeal mixture was the sole food for the first 3 days; however, no additional water was introduced into the egg mixture and the eggs were allowed to slowly desiccate. Fresh oatmeal was then added to the dish. However, the dried egg mixture remained in all dishes during the total exposure. These beetles were dissected after 20 days and all cysticercoids were counted.

On the 27th day after infection with the single cysticercoid each rat was weighed again and then sacrificed; the small intestine was opened and examined for a tapeworm. When one was found, its place of attachment, length, and wet weight were recorded, and the worm was placed in distilled water in a shell vial, stored in the deep freeze, and later freeze-dried for subsequent studies.

All data were analyzed statistically using "Student's" t-test for probability of significance of differences between means.

RESULTS

Sixty-nine rats were used in these experiments; of these, four died of unknown causes before they were tested for eggs at the end of the 20 days. Eggs were found in the feces of 56 of the 65 (86 percent) surviving rats. Thirty-one rats in the normal and starved series survived (100 percent) until they were killed for examination; however, only 17 of 25 (68 percent) cannulated rats survived the 7-day experimental period. None of the rats which died were examined for tapeworms; therefore, in the subsequent results only the animals living until sacrificed will be discussed. Normal rats and bileless rats were maintained at all times on Laboratory Chow (Ralston Purina Company, General Offices, St. Louis, Missouri) available ad libitum.

Tapeworms were recovered at autopsy 7 days after the start of the experimental period. In 15 normal rats 15 worms (100 percent) were found, 11 (69 percent) worms were recovered from 16 starved hosts, and 11 (65 percent) from 17 bileless hosts. The normal rats and a majority of the bileless rats continued in excellent health during the 7 days; the starved rats, obviously, showed signs of inanition and gave evidence of coat roughness. They were still vigorous, however, and seemed only slightly less responsive than the well-fed controls.

Rats in the normal series averaged 277 g at the start of the 7-day period, and 296 g at the end, an average gain of 19 ± 5.2 g (standard error of mean). In the bileless series the initial average weight was 269 g, and the terminal weight 285 g, a gain in average weight of 16 ± 11.0 g. In the starved series the average weight at the start was 279 g, and 228 g terminally, which represents a loss in average weight of 51 ± 3.6 g (table I).

Weight changes in experimental hosts, place of attachment of the tapeworms, length, and wet weight of tapeworms are given in table I. It is evident, from the table and as mentioned above that normal and bileless hosts gained nearly the same weight in grams, whereas the starved hosts lost. Weight changes between normal and bileless hosts were not significantly different, but they were, at "p" values of less than 1 percent, between normal and starved, and bileless and starved. Comparisons

TABLE I. *Comparison of rat hosts, and site, length, and wet weight of tapeworms in normal, starved, and bileless hosts, with standard error of mean.*

Host	Weight changes of 250 to 270-g rats during 7 days, in grams		Place of attachment of tapeworm posterior to pyloric valves, in cm		Lengths of tapeworms, in cm		Wet weight of tapeworms, in mg	
	Mean	p values	Mean	p values	Mean	p values	Mean	p values
Normal	+ 19 ± 5.2	< 0.01*	17 ± 2.5	> 0.50*	67 ± 4.1	> 0.10*	1165 ± 11	< 0.01*
Starved	− 51 ± 3.6	< 0.01**	15 ± 3.5	< 0.02**	61 ± 7.4	< 0.01**	230 ± 43	< 0.02**
Bileless (cannulated)	+ 16 ± 11.0	> 0.50***	29 ± 4.3	< 0.02***	26 ± 2.7	< 0.01***	109 ± 19	< 0.01***

* Probability of significant difference between normal and starved hosts.
** Probability of significant difference between starved and bileless hosts.
*** Probability of significant difference between normal and bileless hosts.

between attachment points of tapeworms in the small intestine revealed that worms in normal and starved hosts were situated at the same level, about 15 to 17 cm behind the pyloric valve; on the other hand, worms in bileless hosts were found attached nearly 30 cm posterior to the pyloric valve; this more posterior location in bileless hosts was significantly different ("p" values less than 2 percent) from the locations in the other types of hosts. It was rather surprising to find that worms in normal and starved hosts were statistically equivalent in length, but that worms in bileless hosts were shorter than those in either normal or starved hosts. Striking differences can be seen in table I for wet weights. The mean weight of 1165 ± 11 mg for worms in normal hosts was significantly different, "p" values at less than the 1 percent level, from the 230 ± 43 mg for worms from starved hosts, and from the 109 ± 19 mg for worms from bileless hosts; mean wet weight differences of tapeworms from starved and from bileless hosts were significantly different ("p" values less than 2 percent). Proglottids were shed en masse in some bileless and starved hosts, and were found free in the gut lumen at autopsy. The shed proglottids from a bileless host were filled with fat-like vacuoles and were opaque marginally; and the eggs were irregular in shape and lacked the normal internal morphology (cf. also Goodchild, 1958).

In table II comparisons are made between daily egg output by tapeworms in

TABLE II. *Comparison of egg output and egg infectivity of tapeworms grown in normal, starved, and bileness hosts with standard error of mean.*

Host	Daily egg production by tapeworms		No. tapeworm eggs fed daily to beetles		Cysticercoids recovered per beetle	
	Mean	p values	Mean	p values	Mean	p values
Normal	130,800 ± 6,000	< 0.01*	25,000 ± 3,200	> 0.50*	4.7 ± 0.9	< 0.01*
Starved	48,600 ± 11,100	> 0.10**	26,400 ± 10,600	> 0.50**	1.8 ± 0.6	> 0.10**
Bileless (cannulated)	64,700 ± 10,300	< 0.01***	25,200 ± 8,400	> 0.50***	2.4 ± 0.6	< 0.02***

* Probability of significant difference between normal and starved hosts.
** Probability of significant difference between starved and bileless hosts.
*** Probability of significant difference between normal and bileless hosts.

the 3 series, between numbers of eggs fed daily to beetles, and between cysticercoids recovered per beetle. Daily egg counts were made of eggs contained in each day's

collection of feces for 7 days from seven normal rats and from nine starved rats; similar counts were started on eggs in feces of 15 cannulated rats; however, because of deaths in this series, eggs in feces from 15 rats were counted on day 1, from 14 hosts on days 2 and 3, and from 12 rats on days 4, 5, 6, and 7. The seven worms in normal hosts produced an average of 916,000 eggs during the 7 days, nine worms from the starved rats produced an average of 340,200 eggs during 7 days, and worms in bileless hosts had average counts of 453,200 eggs in the same interval. Average daily counts for the 7-day period may be determined from figure 1. In this figure it

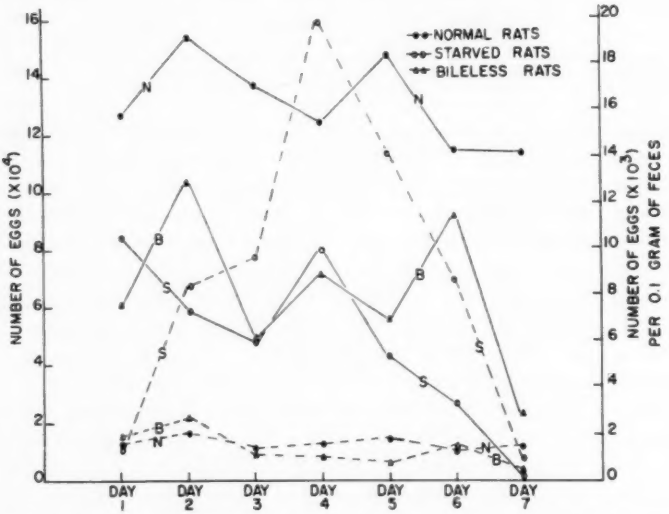

FIGURE 1. Mean daily egg production and egg output per 0.1 g of feces per worm. Solid lines represent total mean egg production and are based on left ordinate. Broken lines represent mean egg output per 0.1 g of feces and are based on right ordinate.

can be seen that egg production remained relatively steady in normally fed hosts, but tended to be depressed to low levels by day 7 in starved and bileless hosts. Daily egg outputs were significantly depressed, "p" values at less than 1 percent levels, when comparisons were made between normal and starved or bileless, but were not significantly different between bileless and starved.

Although beetles in each container were fed similar average numbers of eggs, average numbers of cysticercoids recovered per beetle differed in the three categories. Significantly fewer larvae were recovered in the starved (1.8 ± 0.6) and bileless (2.4 ± 0.6) series as compared to those recovered from eggs from tapeworms in normal hosts (4.7 ± 0.9); yet comparison between cysticercoid recoveries in the starved and bileless series revealed lack of a significant difference: "p" values of significance were at the 1 percent level between normal and starved, at the 2 percent level between normal and bileless, but at the 10 percent level between starved and bileless. From a total of 324,400 eggs from normal hosts 127 surviving beetles yielded 600 cysticercoids; from 369,100 eggs from starved hosts 139 surviving beetles yielded 254 cysticercoids; and from a total of 327,700 eggs from bileless hosts 127 surviving beetles yielded 303 cysticercoids. Table III shows the daily counts of

TABLE III. *Number of eggs fed daily to beetles and number of beetles examined after 20 days with average recovery of cysticercoids per beetle.*

Host	Days						
	1	2	3	4	5	6	7
Normal	55,300 (20 ; 4.4)*	42,000 (19 ; 4.3)	40,400 (10 ; 11.2)	40,800 (20 ; 2.3)	40,000 (20 ; 3.2)	66,900 (19 ; 3.7)	39,000 (19 ; 7.8)
Starved	24,800 (20 ; 2.0)	22,800 (20 ; 1.7)	68,400 (19 ; 2.2)	9,600 (19 ; 1.2)	218,000 (20 ; 5.6)	22,000 (20 ; 0.0)	3,500 (21 ; 0.2)
Bileless	24,200 (18 ; 0.06)	51,000 (10 ; 2.2)	156,300 (20 ; 2.8)	37,600 (20 ; 1.3)	32,000 (20 ; 2.0)	38,000 (20 ; 7.0)	15,800 (19 ; 1.2)

* Numbers in parentheses represent total beetles dissected and average cysticercoids per beetle.

eggs fed to beetles and the number of beetles dissected after 20 days with average recovery of cysticercoids per beetle.

When ratios of eggs fed per beetle to cysticercoids produced per beetle were calculated it was determined that in the normal series approximately 1000 eggs yielded 2 cysticercoids, and in the starved and bileless series 1000 eggs yielded 1 cysticercoid in each. Mean values of cysticercoids recovered per eggs fed were 0.2 percent $\pm$ 0.10 in the normal series, 0.11 percent $\pm$ 0.08 in the starved series, and 0.12 percent $\pm$ 0.15 in the bileless series. The standard error of the mean is excessive in the last series due to an unexplained large deviation on day 6 where 10 beetles in each of two containers had received 22,600 and 15,400 eggs, respectively, and from which 82 (0.36 percent) and 57 (0.37 percent) cysticercoids were recovered, for an average of seven cysticercoids per beetle. Obviously, force-feeding of beetles is impossible, and although techniques of exposure, as well as pre-treatment and post-treatment were identical these results may reflect the ingestion of more oncospheres rather than increased infectivity on day 6. Another unexplained occurrence was the failure of infection of beetles with eggs from bileless hosts on day 1; here 24,200 eggs had been offered to 18 beetles from which after 20 days only one cysticercoid was recovered. These feeding experiments were planned originally to give a possible yes or no answer with respect to oncospheral infectivity, hence the lack of concern with totals of eggs offered to beetles. The period of 7 days is perhaps too short to allow full effects of these experimental alterations to be manifest. It is now believed that to have started egg counts on day 4 after start of treatment and to have continued them for 7 additional days might have yielded the yes or no answer sought.

In table IV is given the mean daily output in grams of feces from normal, starved,

TABLE IV. *Comparison of mean fecal (with standard error of mean) and egg output from normal, starved, and bileness rat hosts harboring single rat tapeworms,* Hymenolepis diminuta.

Hosts	Days						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Normal	8.0 $\pm$ 1.3* (1,590)**	7.6 $\pm$ 1.2 (2,030)	10.0 $\pm$ 1.6 (1,370)	8.3 $\pm$ 1.4 (1,490)	8.3 $\pm$ 0.8 (1,780)	9.1 $\pm$ 1.2 (1,250)	7.9 $\pm$ 1.4 (1,430)
Starved	5.5 $\pm$ 1.1 (1,530)	0.7 $\pm$ 0.1 (8,410)	0.5 $\pm$ 0.2 (9,620)	0.4 $\pm$ 0.1 (19,830)	0.3 $\pm$ 0.1 (14,200)	0.3 $\pm$ 0.1 (8,670)	0.1 $\pm$ 0.05 (900)
Bileless	3.2 $\pm$ 0.4 (1,880)	4.0 $\pm$ 0.7 (2,590)	4.9 $\pm$ 1.0 (990)	7.3 $\pm$ 1.4 (970)	7.6 $\pm$ 1.5 (730)	7.0 $\pm$ 1.0 (1,310)	7.8 $\pm$ 1.3 (290)

* Mean weight $\pm$ S.E.$_m$ in grams.
** Numbers in parentheses are eggs per 0.1 g feces.

and bileless rat hosts harboring single rat tapeworms. For normal rats the mean daily output ranged between 7.6 g and 10.0 g; in starved rats there was a gradual

decrease in fecal output during the 7 days, reaching a minimal mean value of 0.1 g on day 7; in bileless hosts daily output rates were depressed until day 4 following the operation. Beyond this time bileless rats had output rates equivalent to those in normal rats. It is probable that the 3 g difference in weight gains between normal and bileless rats, noted above, may be due to the early non-eating period following the operation. The correctness of this contention may be strengthened when output rates between starved and bileless hosts on day 1 are compared. On this day the starved hosts produced, on the average, 2.3 g more feces than did the bileless hosts which had food before them continuously. Large standard errors in the bileless series are due, of course, to large deviations from the means in a few of the hosts; one rat in this series produced 22 g of feces during 1 day, whereas a few produced none. Two such hosts, which produced no feces on the day preceding death, are included in the averages. The mean number of eggs per 0.1 g of feces is also given in table IV; however, standard errors of these means were not calculated.

DISCUSSION

The most important single consideration emerging from this, and the earlier study (Goodchild, 1958), is that tapeworms in bileless hosts, feeding to repletion, responded morphologically and functionally as though they were in hosts totally deprived of food, or else they exhibited effects even more adverse than in starved hosts. Starvation had greater effects upon rat hosts than did biliary cannulation, since differences between weight losses in starved and either bileless or normal hosts (but not between the latter) were statistically significant; however, effects upon tapeworms were not always correlated with effects of treatments upon hosts. For example, tapeworms were not significantly shorter when comparisons were made between normal and starved hosts, whereas they were when comparisons were made between normal and bileless, and bileless and starved; yet weight losses in tapeworms from bileless and starved hosts were significantly different from those in worms from normal hosts. The obvious implication here is that worms in starved hosts were long and attenuated, whereas in bileless rats they were both shorter and weighed less. In other words, tapeworms in hosts completely deprived of food were longer and heavier than were those in bileless hosts which had fed to repletion, and which had increased in weight at normal rates.

Effects of short-term starvation upon hymenolepids have been investigated by Daugherty (1956), and by Read and Rothman (1957). Daugherty found that worms from hosts starved for 24 hours had depleted glycogen stores in the anterior end (to about 15 mg per g as compared to a normal 100 mg per g) and that glycogenesis, from an in vitro glucose substrate, was accomplished at higher rates than it was in unstarved worms; contrariwise, exposure of starved worms in vitro to a pyruvate substrate failed to yield evidence of glycogenesis. Read and Rothman (loc. cit.) fed infected rats, after they had been starved for 24 hours, 0.5 g starch and then killed them and harvested the worms after an additional 4, 24, and 48 hours. Progressive losses in wet weight of worms in the presence of exogenous starch, and an augmented in vitro glycogenetic efficiency in utilization of exogenous glucose, by *H. diminuta*, but not by *H. citelli*, were found to occur. In the present study, as just mentioned, weight losses of worms were noted in both starved and bileless hosts; although rates of glycogenesis cannot be determined in these worms, they have been freeze-dried and subsequently will be analyzed to determine quantities of carbohy-

drates, fats and proteins.

Another interesting analysis was the site of attachment of the scolex. Between normal and starved hosts no significant difference was found in place of attachment. In bileless hosts, however, when compared with either normal or starved hosts, tapeworms were significantly more posteriorly located within the small intestine. This finding supports the contention that acidity-alkalinity, or other environmental optima, are influenced by the secretion of bile and that tapeworms move posteriorly to reach new zones of optima. Adult tapeworms have the capacity to move within the small intestine (Chandler, 1939; Goodchild, 1958; Foster and Daugherty, 1959), so posterior migration was neither startling nor unexpected.

Rothman (1958, 1959) has reported an in vitro inhibition of anaerobic metabolism in various species of tapeworms with whole bile, bile salts, sodium taurocholate, sodium glycocholate, and sodium cholate. The inhibition was pH sensitive and could be reversed by washing worms free of the biliary additives; furthermore, the inhibition may not be solely related to decreased surface tension due to biliary components since digitonin and Tween 80, surface tension depressants, behaved somewhat differently from the bile salts. Rothman (1959) commented that the seeming essential in vivo nature of bile (Goodchild, 1958) may be due to secondary factors rather than "to the direct effects of the bile alone." It is hoped that with additional work the differences between these in vitro and in vivo results may be resolved.

Rates of egg production in starved and bileless hosts are more difficult to analyze. In both types of hosts significantly fewer eggs were produced than in normal hosts, but there was no significant difference in egg output between starved and bileless rats. Single tapeworms in normal rats produced eggs at a daily rate of between about 115,000 and 150,000, which is in agreement with results reported by Beck (1951) for the same host; whereas, even from the first day in the present experiments egg counts were depressed (60,000 for bileless and 84,000 for starved hosts). Although fluctuations occurred, reaching highs of 102,000 eggs (day 2) in bileless hosts, and 78,500 eggs (day 4) in starved hosts, the total 7 day outputs were only one-third (from starved rats) to one-half (from bileless rats) the normal rate. One fact seems evident from this study: this is a residual normal effect of about 4 to 6 days' duration, at which time precipitous declines in egg output occurred in each experimental group. In the starved hosts elimination of eggs has essentially ceased by day 7 (900 eggs for the day); by extrapolation, egg output would have terminated in the bileless hosts by day 8. Even this lengthy persistence of egg production was unexpected since changes in gravid proglottids were evident before the end of the 7-day period. These changes included loss of normal egg structure and altered opacity of the proglottid. Similar findings for proglottids were reported for slightly longer periods in bileless hosts by Goodchild (1958).

The finding of structurally normal cysticercoids in beetles fed eggs collected from the two types of experimental hosts on the seventh day was also somewhat unexpected, since eggs within proglottids at that time were atypical. Whether this signifies that clusters of normal eggs have escaped detection in morphological searches of the proglottid, or whether normal eggs remain for varying periods of time in the gut before elimination is not known.

Fecal output from normal, starved, and bileless hosts, and egg counts in equivalent weights of feces gave insight into the response of the hosts and effects upon the parasites. It will be seen (table III) that normal rats had a rather steady output of

about 8.0 to 9.0 g of feces per day. In the starved hosts a gradual decline in daily output occurred during the 7-day period. All rats in this series produced feces on days 1 and 2; on days 3, 4, 5, 6 one rat (but not the same one) failed to produce any pellets; on day 7 four of the nine rats failed to produce feces, and the maximum weight on day 7 was 0.4 g. Bileless rats, as might be expected, failed to eat at the usual rate during the first 3 days following the serious operation; however, by day 4 the mean fecal output had increased to a near-normal level; for example, the output in bileless hosts on day 7 exceeded the output on day 2 in the normal series. Larger standard errors were calculated for some days in the bileless series since all rats did not recover at the same rate or to the same degree. In fact, the largest daily amounts were observed on days 4 and 5 (20.6 g and 22.0 g, respectively) in 1 host (E-22) in the bileless series. Egg counts per unit weight of feces were rather constant in the normal series, ranging from 1,250 to 2,030 per 0.1 g of feces. In the starved rats counts were strikingly increased on days 2, 3, 4, 5, 6, and maxima occurred on days 4 and 5. It seems obvious that these high counts were due to detachment and elimination of large segments of proglottids. In some hosts, as mentioned above, detached lengths of proglottids were recovered in the small intestine at autopsy. In others, proglottids were present in the feces and, in fact, constituted a large fraction of the fecal weight for that day. In the bileless series, on the other hand, a 1-day increase in egg output occurred on day 2, and henceforth a general decline occurred; by day 7 egg output had practically ceased.

There are other comparisons which could be made between hosts and parasites in the 3 series; however, many of these comparisons have been largely anticipated in a previous paper (Goodchild, 1958) and will not be further elaborated upon here.

SUMMARY

Sixty-nine Wistar rats were fed single cysticercoids of the rat tapeworm, *Hymenolepis diminuta*. Fifty-six revealed the presence of tapeworm eggs in the feces from a total of 65 rats which lived for 20 days after infection.

On day 20 after infection 16 infected rats were starved, 25 infected ones had operations performed to reroute bile to the caecum via a cannulary by-pass and were subsequently retained on a normal diet, and 15 infected rats remained on a normal diet. Feces collected from 31 rats (9 starved, 15 bileless, and 7 normal) during the next 7 days were subjected to egg counts, and random samples of daily collections of eggs were offered to starved flour beetles, *Tribolium confusum*.

After the 7-day experimental period all rats were sacrificed, and place of attachment, length, and wet weights of recovered tapeworms were recorded. Twenty days after tapeworm eggs had been offered to beetles they were sacrificed and all cysticercoids counted.

Statistical analysis showed that starved rats lost weight at rates significantly different from bileless or normal; bileless and normal rats gained weight at the same rate; tapeworms in bileless hosts were more posteriorly located in the small intestine; worms from starved rats were as long as from normal hosts, but were shorter from bileless hosts; worms in bileless and starved rats weighed less than worms from normal hosts; egg output by tapeworms in bileless and starved hosts was equal, but was less than from normal hosts; and finally, although beetles had received the same number of eggs, significantly different recovery rates of cysticercoids occurred from eggs of the experimental groups.

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RESEARCH NOTE

HELMINTHS OF THE YELLOWTHROAT, *GEOTHLYPIS TRICHAS*, DURING MIGRATION.

A study was made of the helminth parasites of 40 specimens of the yellowthroat, *Geothlypis trichas* (L.). These yellowthroats were collected at the base of a television transmission tower located 10 miles northwest of Durham, N. C., on October 1 and 3, 1957. The birds were migrating southward and had collided with the tower during the night. When collected, they had been dead less than 8 hours, or were still alive but badly injured. Some birds were examined immediately and others were frozen for later examination.

Of 15 females, 15 males, and 10 immature specimens examined for parasites, 8 females, 9 males, and 3 immature birds harbored helminths. Parasites included two species of trematodes from 5 birds (12.5 percent), two species of cestodes from 4 birds (10 percent), two species of acanthocephalans from 5 birds (12.5 percent), and four species of nematodes from 12 birds (30 percent). The following species were represented: (1) TREMATODA: *Brachylecithum americanum* Denton, 1945, was recovered from the smaller bile ducts of the livers of 4 birds, and *Zonorchis alveyi* (Martin and Gee, 1949), from the gall bladder of 1 bird. (2) CESTODA: *Anonchotaenia* sp. was recovered from the small intestines of 2 birds, and *Biuterina* sp., from the small intestines of 2 birds. (3) ACANTHOCEPHALA: *Apororhynchus amphistomi* Byrd and Denton, 1949, was recovered from the cloacal regions of 5 birds, and *Mediorhynchus robustus* Van Cleave, 1916, from the small intestine of 1 bird. (4) NEMATODA: *Oxyspirura pusillae* Wehr and Hwang, 1957, was recovered from beneath the eyelid of 1 bird; *Oxyspirura petrowi* Skrjabin, 1929, from beneath the eyelids and nictitating membranes (and in one case, imbedded in the eye muscles) of 5 birds; *Diplotriaena* sp. (immature), from the pericardial cavity of 1 bird; and *Ornithofilaria inornata* Anderson, 1956, from the abdominal cavities and mesenteries of 5 birds. Only two species, *Diplotriaena* sp. and *Oxyspirura petrowi*, were found in immature birds.

Generally, the number of helminths per infected bird was low. Certain species were represented by but one specimen (*M. robustus, O. pusillae, Diplotriaena* sp.). The maximum numbers of specimens from a single host were as follows: *B. americanum*, 10; *Biuterina* sp., 13; *A. amphistomi*, 12; and *O. petrowi*, 17.

The following seven species have not been reported previously from the yellowthroat: *Brachylecithum americanum, Zonorchis alveyi, Apororhynchus amphistomi, Mediorhynchus robustus, Oxyspirura petrowi, O. pusillae*, and *Ornithofilaria inornata*.—MARY JANE WELLS AND WANDA SANBORN HUNTER, *Department of Zoology, Duke University, Durham, North Carolina.*

A REDESCRIPTION OF *AVITELLINA ARCTICA* KOLMAKOV, 1938 (ANOPLOCEPHALIDAE: THYSANOSOMINAE), FROM *RANGIFER ARCTICUS ARCTICUS* IN NORTHERN CANADA

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While studying the intestinal parasites of the barren-ground caribou (*Rangifer arcticus arcticus*) a species of the genus *Avitellina* was found, which appeared to closely resemble the published description of *A. arctica* Kolmakov, 1938. Species of this genus have been reported from Italy, southeastern Europe, north and south Africa, India and Russia, where they are fairly common parasites of ruminant hosts. However, the writer was unable to find any previous records of this genus from the western hemisphere. This, therefore, represents a new record of this genus in North America.

Because of the somewhat vague description of *A. arctica* given by Kolmakov (1938) and the fact that no other descriptions of this species exist, it was felt that a redescription of the species would be valuable.

Two complete worms were available for study. These specimens were supplemented by stained and mounted examples supplied by Dr. Allen McIntosh of the U.S.D.A., which had been collected by Dr. C. D. H. Clarke from the same host in the Thelon Game Sanctuary, N.W.T., in 1936 to 1937. The worms were fixed in 10 percent formalin and stained with Ehrlich's haematoxylin. The measurements given are the ranges or averages of 10 measurements taken from representative portions of the strobila.

DESCRIPTION

Total length of one entire specimen about 270 cm. Scolex unarmed (fig. 1), somewhat goblet-shaped, equipped with 4 suckers; diameter of suckers 0.26 mm, maximum width scolex 1.02 mm, leads into narrow neck. Neck region indistinctly segmented, dorsal excretory canal medial to ventral excretory canal and visible in this region. Neck leads into strobila where segmentation is readily apparent, genital primordia distinguishable in this region. This area followed by region where male genitalia mature (fig. 2), width about 1.5 mm to 2.0 mm. Width, early gravid segments, about 3.0 mm, mature gravid segments about 1.0 mm.

Musculature on cross-section consists of thin layer of longitudinal muscles just below cuticle and a layer under subcuticle with transverse-connecting fibres in cortical region. Cortical parenchyma more closely meshed than medullary parenchyma (fig. 8).

Excretory system of 2 small dorsal canals visible in neck region of strobila medial to 2 larger ventral canals which extend length of strobila. Longitudinal nerves lateral to ventral excretory canals, midway between latter and margin of strobila.

Genital pores alternate irregularly between segments. Male genitalia consist of cirrus sacs which open by way of genital pore, slightly anterior to vagina; irregularly either dorsal or ventral to vagina. Cirrus sacs measure 0.153 to 0.170 mm long, contain a cirrus, in some segments extended to exterior. Cirrus sac leads medially into coiled vas deferens and then to testis (fig. 3). Testes in 4 rows in each segment; 2 rows, 8 to 9 testes each, medial to excretory canals; 2 rows, 4 to 5 testes each, lateral to excretory canals, between it and longitudinal nerve, testes measure 0.06 to 0.08 mm in diameter (fig. 4).

Female genitalia in mature segment consist of glandular, terminal, fusiform portion of vagina opening at genital pore posterior to male cirrus (fig. 3). This portion about 0.153 mm

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long leads into thinner portion which in turn leads dorsally to oval receptaculum seminis. Receptaculum seminis thin-walled oval or flask-shaped organ, about 0.128 mm long and 0.11 mm wide, situated midway between parauterine organ and excretory vessel on poral side of segment. From receptaculum seminis oviduct proceeds to ovary. Ovary about 0.102 mm long and 0.068 wide, situated close to parauterine organ on poral side. From ovary, oviduct proceeds to parauterine organ (fig. 5). Parauterine organs in male mature segment simple, sac-like about 0.3 mm long, disposed in central row in strobila (figs. 6 and 7). Undergo considerable change with increase in maturity. In female, mature segments become wedge-shaped with folds (figs. 9 and 10), later central portion grows out forming a spherical, bladder-like sac containing up to 12 pear-shaped egg capsules (figs. 11 and 12). Each sphere capped by a wedge-shaped darkly staining anterior portion, so called inter-proglottidal sheets or fibrous pads. These organs may alternate with each other from side to side or lie one above the other in strobila (fig. 11). Ova in capsules, spherical 0.018 to 0.022 mm in diameter.

Hosts: *Rangifer tarandus uralensis*
Capreolus pygargi
Rangifer arcticus arcticus

Locality: Polar Ural, Obdorsk Region, West Siberia 67°N. Lat. U.S.S.R.
Thelon Game Sanctuary, N.W.T. 64° Lat. Canada.

Habitat: Small intestine

DISCUSSION

Avitellina arctica most closely resembles *A. centripunctata*. However, the writer's specimens differ from the description (Woodland, 1927) of *A. centripunctata* in a number of respects. *A. centripunctata* has only 2 testes per segment in the outermost row and 4 testes in the innermost row, while *A. arctica* has 4 or 5 testes in the external row and 8 or 9 testes in the internal row. The position of the cirrus sacs is variable in *A. arctica* and not constantly dorsal to the vulva on the right side and ventral to it on the left side as in *A. centripunctata*. The mature parauterine organ is pear-shaped in *A. centripunctata* while in *A. arctica* it is spherical. The parauterine organs in *A. centripunctata* develop definite wide openings into the large ventral excretory canals and pour into them egg-filled capsules which in turn liberate eggs into these canals; no such openings are discernible in the writer's material nor are ova present in the excretory canals even in the most mature segments.

The 2 anterior ends in the writer's specimens both have a curious structure in that there is a goblet-shaped scolex borne on a short thin neck, which abruptly leads into the strobila proper (fig. 1). The one scolex in McIntosh's material is more typically avitelline, is not globular, and does not apparently lead abruptly into the strobila. Whether this variation is of taxonomic importance is debatable; the writer considers it to be merely a developmental anomaly, since these 2 scolices were both obtained from the same host animal.

As Spasski (1951) has pointed out, there appear to be definite host and ecological differences between these 2 species. *A. centripunctata* is a parasite of horned ruminants (sheep, cattle and antelopes) in temperate and torrid zones, while *A. arctica* has only been described from Cervidae in boreal regions. In Russia neither *A. centripunctata* nor *A. arctica* have been obtained from sheep in boreal regions. Yet, as Spasski (1951) points out, the ranges of *Capreolus* (host of *A. arctica*) and that of sheep in these northern regions overlap. Thus infection of sheep with *A. arctica* could conceivably occur if no barriers attributable to host-specificity were present. As infection does not appear to occur one must conclude that these barriers do indeed exist and that *A. arctica* is host-specific for Cervidae. In turn, because *A. centripunctata* is not found in sheep in these regions it seems reasonable to conclude that its ecological requirements do not permit of its occupying a niche as far north as the one in which *A. arctica* is found.

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Description of Plates

Key to lettering: tv, terminal portion of vagina; cs, cirrus sac; ln, longitudinal nerve; ot, outer row of testes; it, inner row of testes; ec, excretory canal; rs, receptaculum seminis; o, ovary; p, parauterine organ; vd, vas deferens.

Plate I

Figure 1. Scolex and neck.
Figure 2. Mature segments.
Figure 3. Detail of cirrus sac and terminal portion of vagina.
Figure 4. Detail mature segments.
Figure 5. Detail of receptaculum seminis, ovary and parauterine organ.
Figure 6. Detail of mature segments.
Figure 7. Detail of mature segments.
Figure 8. Cross section, mature segments.

Plate II

Figure 9. Early gravid segments.
Figure 10. Detail, early gravid segments.
Figure 11. Gravid segments.
Figure 12. Detail gravid segments.

PLATE I

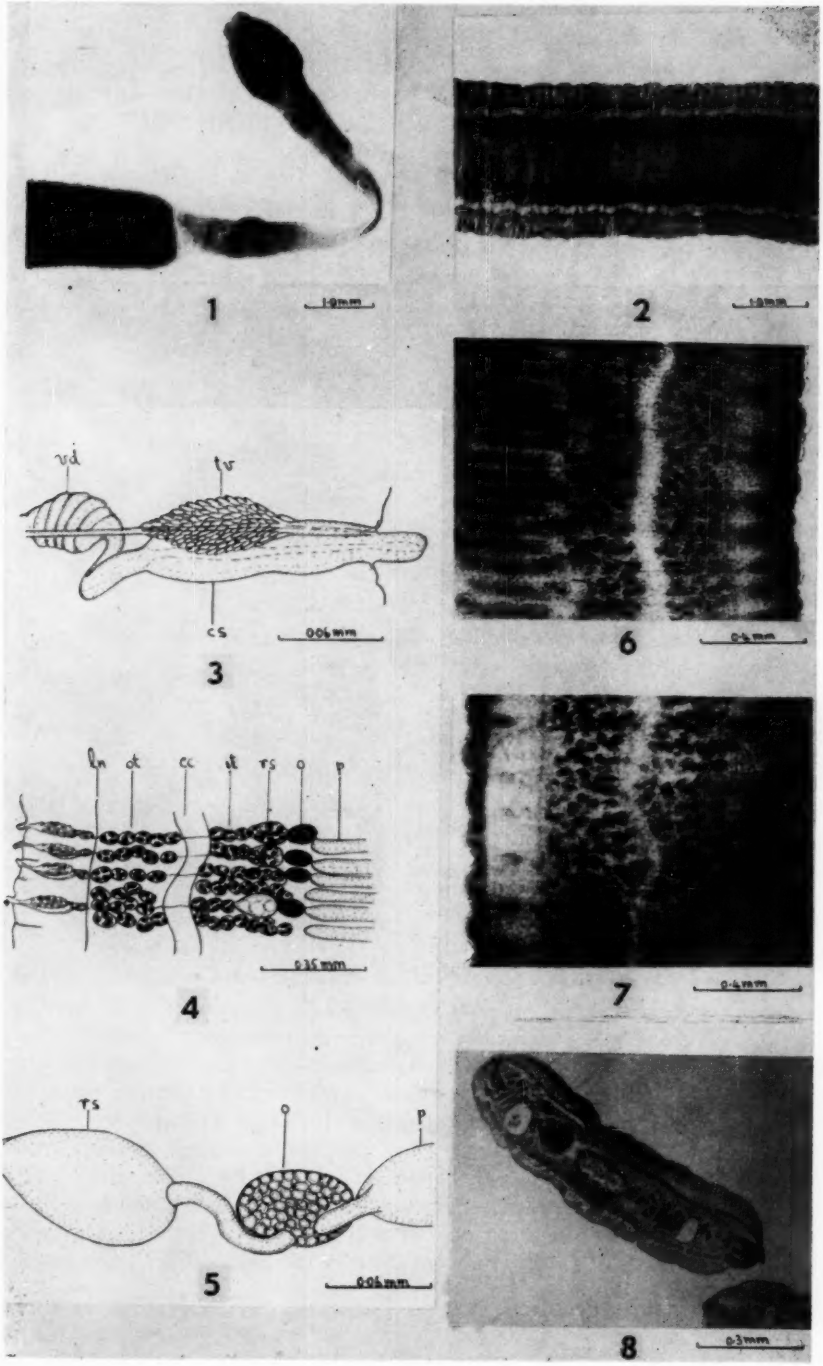
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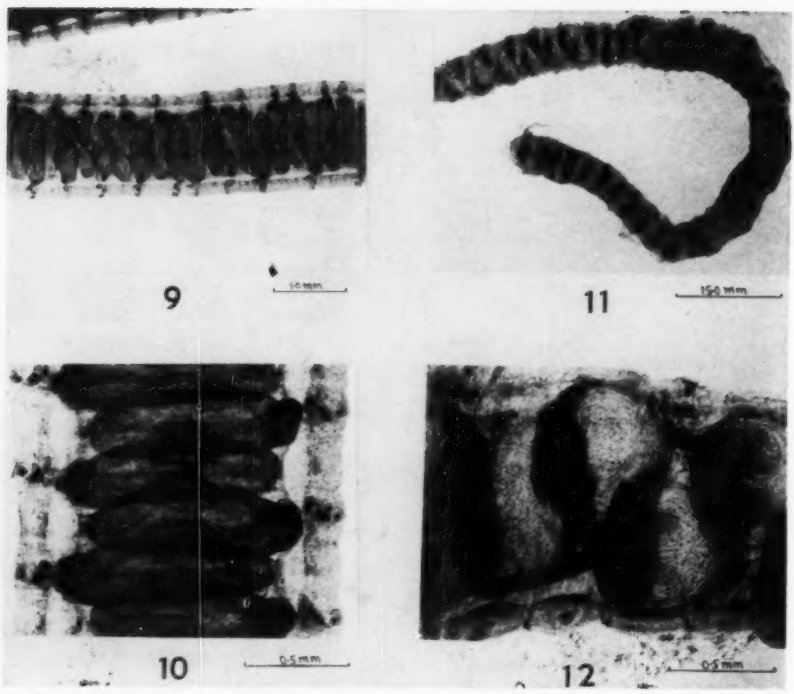
PLATE II



STUDIES ON *SCHISTOSOMA INCOGNITUM* CHANDLER, 1926
II. ON THE LIFE HISTORY OF THE BLOOD FLUKE

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Though *Schistosoma incognitum* is a common blood fluke of pigs and dogs in India and Rao (1937) has indicated its pathogenic significance, there has been little study on the biology of the parasite. Rao studied only briefly the miracidium of *S. incognitum* obtained from the feces of a dog. While investigating the possibility of the spread of human schistosomiasis in India, Khaw (1947) recovered adults of *S. incognitum* by the passage of cercariae, obtained from naturally infected *Lymnaea luteola* var. *succinea* or *ovalis*, through rabbits. He did not work out any more details, not even the morphology of the cercaria. A detailed study of the life cycle of the parasite forms the subject of the present paper. An abstract of the present paper was published in the Proceedings of the Indian Science Congress (Sinha and Srivastava, 1954). In the first paper of the series, the synonymy and morphology of *S. incognitum* were dealt with (Sinha and Srivastava, 1956).

MATERIALS AND METHODS

Parasites were available from the pork market and from donor animals maintained in the laboratory. The egg, miracidium and cercaria were always from a known source or identity was later confirmed by experimental infection. The definitive and intermediate hosts were both bred in the laboratory for experimental infection. Those which were obtained from outside were kept under observation before they were declared free from infection.

Concentration of miracidia was facilitated by devising a certain procedure. The feces of an infected pig was well diluted with cold water below 20 C, strained through fine mesh, sedimented and the supernatant was then decanted. After several such washings with cold water the sediment was collected in a conical flask which was then filled with water at room temperature up to the neck. The flask was then covered with black cloth, leaving only a short water column at the top exposed to bright light. Miracidia, being phototropic, all crowded into the lighted part. This method was quite effective in determining even light infections. Fecal examination often did not detect eggs in light infections. Exposure of snails to miracidia was done individually or in batches of few with few miracidia in a small quantity of water in each test tube.

All measurements are in millimeters unless otherwise indicated.

The Egg

Development of the egg. The egg in the uterus of the female worms contains a large nucleus with homogeneous cytoplasm and a number of yolk cells. While the uterine eggs showed little development of the embryo, the eggs present in the wall of the intestine were at different stages of development. Some were at the morula stage and others contained developing and mature miricidia. The eggs recovered from the scraping of the intestinal wall and the intestinal contents always harbored fully mature miracidia. The mature egg passed in the feces contains a fully formed miracidium. The miracidium often shows movement. The cilia move in a wavy rhythm, the wave starting from the cilia near the anterior end. The flame cells are active. Sometimes the miracidium rotates on its long axis. The mira-

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cidium may change its position and come to lie with its anterior end directed away from the spine. The movements of the miracidium are increased by heat and light. The "shoulders" observed by Rao (1937) in the miracidium inside the egg shell were not observed.

Hatching. When the eggs are placed in water they swell up slightly and the movements of the miracidium are accelerated. A transverse rupture appears in the middle third of the egg through which the miracidium escapes into the surrounding water. The eggs begin to hatch about 10 minutes to 1 hour after the feces are well diluted, and the hatching of all the eggs is completed within 2 to 3 days, variations in temperature markedly affecting the time taken by the eggs to hatch.

The Miracidium

Movements. The swimming activity of the miracidia increases with temperature and light. The faster they move the more elongated and narrower they become, at times attaining a size double their ordinary length. Ordinarily, they move in a straight course revolving counter-clockwise on their long axis unless attracted by the presence of a suitable molluscan host. Experiments were performed to determine the level of water where the maximum number of miracidia congregate. Two graduated cylinders were three-fourths filled with water and 50 miracidia placed in each of them. Most of the larvae crowded in the top 1-inch layer and the rest were found distributed at lower levels. The deeper the level the fewer the number of miracidia present. This behavior of the miracidia of *S. incognitum* is similar to that of *S. japonicum* and *S. mansoni* observed in similar experiments by Faust and Meleny (1924).

Morphology. (plate I) The miracidia are pyriform in shape as seen in fixed specimens. In the swimming condition, however, the body becomes elongated with a slight constriction in the middle. The size of the miracidium, based on measurements of 60 fixed specimens, was 0.123 to 0.168 in length and 0.058 to 0.077 in breadth, with the maximum breadth occurring across the base of the anterior conical portion.

The outer surface of the body is covered with cilia arranged on the epidermal cells. Each cilium arises from a basal body in the epidermal cell. There are no cilia on the anterior papilla or intercellular spaces. The cilia on the body appear to form 4 belts, the first between the opening of the cephalic gland ducts and the lateral papillae. The second extends from the opening of the lateral gland ducts anteriorly to slightly behind the middle of the body posteriorly. The third belt extends posteriorly up to the opening of the excretory pore. The last one extends to the posterior end. The narrow spaces between these belts are without any cilia. The rows of ciliary patches in these spaces described in the miracidia of certain schistosomes (Faust, 1924; Price, 1931; Gordon et al, 1934, and Rao, 1934) were not observed in the miracidia of *S. incognitum* either by Rao (1937) or the present authors. The cilia are generally of the same size except in the first belt. They are longest near the lateral papillae and gradually become smaller towards the opening of the cephalic glands at the base of the anterior papillae. They measure from 0.003 to 0.01 in length. The cilia on the rest of the body measure on an average 0.01 in length. The cilia in the first belt are set in motion, and are followed by those of the posterior belts. It is also this first belt of cilia which remains active up to the last, while the cilia in the posterior belts cease to vibrate before the death of the

miracidium.

The flat epidermal cells are 21 in number (plate I, fig. 2). They are arranged in 4 transverse rows coinciding with the position of the ciliary belts. The first row of epidermal cells consists of six cells. They are somewhat triangular in shape, their apices being directed towards the apical papilla and their bases extending to the level of the lateral papillae. The second row of epidermal cells consists of 8 cells. These are larger than the rest and are rectangular in shape with rounded corners. The third row has 4 epidermal cells which are almost square in shape with rounded corners. The last row consists of 3 epidermal cells, cuneiform in appearance with their blunt corners directed posteriorly. The nonciliate belts mark the areas where there are no epidermal cells and the subepithelium is exposed. The miracidia at different states of contraction show constrictions at different levels corresponding to the spaces between the transverse rows of epithelial cells.

There are small lateral papillae, 1 on either side situated just above the opening of the ducts of the lateral glands in the miracidia of *S. incognitum*. The primitive gut is sac-like, filled with coarse oxyphilic granules. It extends from the apical papilla to about four-fifths the length of the cephalic glands and reaches to near the nerve mass. The oral aperture is a simple opening. The gut contains 4 nuclei which are situated in the sacular end. Rao (1937) has not referred to the presence of any nuclei in the gut of the miracidium of *S. incognitum*. According to him the cephalic glands in the miracidia of *S. incognitum* are twice as long as the gut, whereas we find the latter to be only four-fifths the length of the cephalic glands. A pair of cephalic glands is situated 1 on each side of and ventral to the gut. They are unicellular and retort shaped with 1 large nucleus in each. They open through wide ducts at the base of the apical papilla. The glands are full of coarse acidophilic granules. They extend through the anterior third of the miracidium. Their length varies from 0.045 to 0.065 (average 0.052).

The lateral glands are situated side by side ventral to the nerve mass and behind the cephalic glands. Their ducts open at the sides in the space between the first and second rows of epidermal cells. Occasionally droplets were seen at the opening of these ducts, which might be secretions from the glands. These glands have been described by Rao (1937) as posterior secretory glands with their ducts opening on short papillae at the region of the so called "shoulder." The nervous system consists of a large central granular nerve mass situated in the anterior half of the middle third of the body length. The nerve mass lies just behind the gut, overlapping the lateral glands.

The excretory system (plate I, fig. 3) of the miracidia consists of 2 pairs of flame cells arranged in bilateral symmetry, as described by Rao (1937). The germ cells are found inside the body occupying the posterior two-thirds. They are round and have large nucleus. There is no cleavage in the germ cells and they are embedded in a spongy tissue, which may be called the mesenchyma. One or more refractile bodies, probably degenerate germ cells, were observed at the level between the nerve mass and the germ cells. We did not observe refractile bodies around the gut as reported by Rao (1937).

Attack and Penetration of the Snail Host

The miracidia swim about mostly in the top layer of water. If the larvae come near a snail of the particular species, *Lymnaea luteola* var. *australis*, they are im-

mediately attracted and stimulated. They swim briskly in the vicinity of the snail before they attack and may leave it after a short while, repeating this several times at different points before finally settling. Some miracidia do not seem to attack the snail seriously. For penetration they exert great muscular action. The body becomes longer and narrower as if trying to push in and then bulges at the anterior end alternately, thus gradually penetrating the epidermis of the snail. After partial penetration some miracidia voluntarily retreat. Porter (1938) found that sunlight and temperature were the principal conditions influencing their attractions to the snail host as also its freedom from previous infection. This has been confirmed in these studies, and the attack and penetration of the miracidia have also been found possible in the electric light.

The miracidia of *S. incognitum* generally show no marked preference for any particular site of attack, and usually attack any exposed part of the snail. However, the head and the anterior end of the foot are more frequently attacked, the tentacles being attacked less frequently. Some miracidia disappear inside the shell attacking the mantle. They are not found to enter the respiratory pore.

Specificity of the Snail Host

Specimens of several species of snails commonly available locally were exposed to infection of the miracidia of *S. incognitum* in the laboratory. The species of snails tried were *Lymnaea luteola* var. *australis, L. acuminata, Indoplanorbis exustus, Melanoides tuberculatus, Vivipara bengalensis, and Gyraulus* sp. All these species except *L. luteola* var. *australis* proved negative to laboratory infection. In nature, also, only this species serves as the intermediate host of *S. incognitum.* In the course of collection of snails round the year in 2 villages, Rithowra and Sithowra near Bareilly, infections of *S. incognitum* were met with in a total of 68 specimens of *L. luteola* var. *australis* of a total of 3,111 snails examined. The positive snails measured 9.5 to 11.9 in length, 5.5 to 12 in width and 4.5 to 9 in height. In every case the discharged cercariae developed into adult *S. incognitum* in laboratory animals.

The population of *L. luteola* var. *australis* was high during March, April, May and October, November, December, January, and February, low in June and September, and rare in July and August. Natural infections were detected mostly during March, April, and May. All other species of snails collected in large numbers from the same areas always proved to be negative for *S. incognitum.*

Experimental infection with *L. luteola* var. *australis* was successful in the laboratory, and the infected snails discharged cercariae which developed into adult *S. incognitum* in the laboratory animals. Khaw (1947) reported *L. luteola* var. *succinea* or *ovalis* to be the intermediate host of *S. incognitum* in Madras.

In experimental infection a good percentage of snails died before the prepatent period was completed. Out of a total of 953 snails exposed to infection, 317 survived long enough for the possible development of the cercaria. But after the prepatent period only 93 snails were found to discharge cercariae of *S. incognitum.* Except the very young ones, the snails at all ages took up the infection readily. The development of the miracidium into cercaria took a longer period during the winter months than during the summer. In December and January the infection developed in a minimum period of 32 days when the mean daily minimum temperature was 46 F and the mean daily maximum temperature was 71 F, and in May in

22 days with the mean daily minimum temperature of 77 F and the mean daily maximum temperature of 103 F.

Development within the Molluscan Host

The successive developmental stages inside the snails were obtained by dissecting the molluscs at different intervals. In snails dissected 5 to 7 days after infection mother sporocysts (plate II, fig. 1) were present. They were found in the tissues around the esophagus of the snail. They are not readily distinguishable from the tissues in which they develop. Freshly dissected specimens show only slight movements with a little change in shape. At times they develop transverse constrictions temporarily. The mother sporocyst is a more or less tubular body with a very thin wall. Four complete specimens of the mother sporocysts were measured, and their sizes varied from 0.2 to 0.23 in length and 0.023 to 0.026 in breadth. They contain many germ cells and several germ balls which give rise to daughter sporocysts.

Early stages of daughter sporocysts (plate II, fig. 2) are found in good numbers in the digestive gland of the snails 7 to 10 days after infection. They are motile and show worm-like movements with expansion and contraction. They are sausage-shaped and develop transverse constrictions temporarily. Their size varies from 0.055 to 0.075 (average 0.065) in length and 0.014 to 0.021 in breadth. The body wall is hyaline and the cells composing the body contain granular protoplasm. There are many refractile granules scattered throughout the body, but in the central core they are denser. Snails dissected 2 weeks after infection yielded daughter sporocysts (plate II, fig. 3) mainly from the digestive glands. At this stage the worm-like movement is still there and transverse constrictions frequently develop temporarily in the tubular shape. Their sizes vary from 0.115 to 0.285 in length (average 0.2) and 0.021 to 0.28 in breadth. There are many germ cells inside the body but germ balls are still absent. The daughter sporocysts (plate II, fig. 4) dissected out of the digestive glands of the snails 18 days after infection show worm-like movement, the tubular body developing transverse constrictions temporarily. Their size varies from 0.26 to 0.428 (average 0.36) in length and 0.04 to 0.045 in breadth. The body wall is extremely thin and in the absence of the conspicuous germ balls it would be difficult to detect it. Germ balls of varying sizes are present inside the sporocyst. Some of the germ balls take the shape of cercaria with an oval body and a protuberance in the position of the tail. Only a few germ cells are seen. There are no developed cercariae at this stage.

The finally developed sporocysts (plate II, fig. 5) were obtained from snails with 21-day old infection. The periods of development of all these stages were determined in May and June when the mean daily maximum temperature was 101 F and mean daily minimum temperature was 78 F. The mature sporocysts were mainly obtained from the digestive glands. There is no movement at this stage. The body is long and tubular with varying diameters at different levels. The size varies from 0.6 to 0.925 in length (average 0.8) and 0.1 to 0.125 in breadth. Each sporocyst contains several fully developed cercariae. Their presence obscures most of the other contents of the sporocyst. Cercariae which have not yet fully developed are also present. The undeveloped cercariae show differentiation of the anterior and posterior penetration glands and the rami of the tail. The fully developed sporocyst has a birth pore at one end through which the cercariae emerge. In heavily infected snails the production of cercariae is so great that on the 24th day the

whole liver tisue appears to be crowded with cercariae liberated from the sporocyst and at that stage it becomes very difficult to trace out a complete sporocyst. These cercariae appear to be similar to the free-swimming one. The above results are comparable to the findings of Gordon and his coworkers (1934) working with *S. mansoni* and *S. haematobium*. What they described as 3 types of sporocysts are really the developing stages of the daughter sporocysts as observed in the present studies.

Damage Produced in the Snail

Swelling due to the penetration of the miracidia of *S. incognitum* at the seat of the attack especially on the tentacles, was not observed in *L. luteola* var. *australis*. Earlier stages of the daughter sporocyst do not produce any apparent damage in the snail. The digestive gland and the gonads appear to be riddled with innumerable sporocysts and cercariae liberated in the tissue. The liver becomes friable and appears to be of slaty or greenish-brown color, instead of a healthy yellow color. The shell becomes thin and brittle. The presence of so many sporocysts produces pressure atrophy. A large percentage of the liver tissue becomes replaced by these sporocysts. The sporocysts probably absorb nutrition which shorten the life of the snail. Infected snails were found to die easily under unfavorable conditions in the laboratory.

The Free Swimming Cercaria

Movements. The cercariae which emerge from the snail are infective. They enter at the earliest opportunity into the body of a warm-blooded vertebrate, or in its absence die after some time. At whatever level of water the snail may be discharging the cercariae, the emerged larvae take a straight course to the top level of the water without delay. During this progressive movement the furcal rami remain foremost and act as the propeller. The tail stem undulates forming definite nodes and internodes. The body is dragged behind. Very rarely, the cercariae move in an oblique direction. When they reach the top layer of the water, the furcal rami touch the top surface and the cercariae remain hanging at that level with the rami spread out at right angles to the tail (plate I, fig. 10). Gradually the cercariae sink by gravitation, but with slightest effort they regain original position. This rising and falling constantly takes place in the top few mm of water. Thus, all the cercariae in the water remain in the top layer and the water below remains free from them. When the water is disturbed and the cercariae become distributed throughout, they soon swim up to the top layer again. Some time before they die the cercariae become rather lethargic and cannot maintain their position in the top level. They are then found gradually gravitating to the lower level of water, occasionally attempting to rise again. The peculiar behavior of the cercariae of *S. incognitum* remaining at the top layer is of great help in its preliminary identification.

Morphology. (Plate II) The cercaria of *S. incognitum* belongs to the non-ocellate, apharyngeate and brevifurcous group of distome cercariae having an oval body much longer than broad and a bifid tail which is much longer than the body. The body has considerable power of extension and contraction. The dimensions of the body after killing by gentle heat are 0.157 to 0.207 in length and 0.042 to 0.071 in breadth. The average of 100 specimens is 0.178 by 0.054. The tail is weakly attached to the body at its posterior end. The tail stem is round and slightly taper-

ing distally. The tail measures from 0.185 to 0.307 (average 0.281) in length and 0.028 to 0.049 (average 0.038) in breadth. The furcal rami are distinctly constricted off from it. The rami are flat, much shorter than the tail and measure 0.085 to 0.135 (average 0.107) in length.

The surface of the body, tail, and rami are armed throughout with small retrorse spines. The spines on the body are more closely set than those on the tail and furci. The spines on the ventral sucker are comparatively more dense than elsewhere. The anterior end of the head organ, which can be inverted like a pouch, does not bear such spines. There are, however, 5 hollow piercing spines situated on either side of the anterior margin of the head gland, through which the ducts of the penetration glands open.

The head organ is pyriform in shape and measures 0.049 to 0.07 × 0.025 to 0.041 (average 0.059 × 0.035). Its walls are muscular and internally it is filled with parenchymatous cells except the portions occupied by the head gland, the esophagus and the ducts of the penetration glands. The head gland which is fusiform in shape and finely granular in texture is dorsally placed. This organ is very indistinct even with intravitum staining and extends to three-fourths of the length of the head organ. There is no true oral sucker. The ventral sucker is a true sucker, and is situated in the third quarter of the body length at a distance varying from 0.106 to 0.138 (average 0.117) from the anterior end. It is strongly muscular and active. It forms an elevation on the ventral side of the body and is protrusible to a certain extent. Its diameter is 0.019 to 0.023 (average 0.021).

The digestive system consists of the mouth, esophagus, and intestinal ceca. The mouth is situated on the ventral surface slightly anterior to the junction of the head organ and the body wall. It leads posteriorly into a narrow more or less straight esophagus which bifurcates into two small, saccular, distinct intestinal ceca just above the level of the anterior penetration gland. There is no pharynx.

The penetration glands are quite conspicuous and are situated in tandem slightly behind the equator of the body. These are 5 pairs of unicellular glands arranged in 2 bilateral groups. The 2 anterior pairs are distinct from the 3 posterior pairs in their coarsely granular protoplasm and acidophilic reaction. The protoplasm of the 3 posterior pairs is finely granular and basophilic in nature. The nuclei in all the cells are prominent. With intravitum stains the anterior pairs remain unstained but the posterior pairs take up stain presenting a sharp contrast. The penetration glands occupy almost the posterior half of the body. The 2 anterior pairs of glands are more or less boot-shaped and lie slightly anterior to the ventral sucker. The 3 pairs of posterior penetration glands occupy nearly all the space between the anterior penetration glands and the excretory bladder. Each gland has a thick duct which runs anteriorly. The ducts of the glands on either side form 2 bundles which run in a zig-zag course in a bilateral symmetry through the body parenchyma, enter the head organ near its posterior margin and open at the anterior end, each duct through a hollow spine. The secretions oozing out of the spines and collecting at the bases of the spines are distinctly seen in specimens stained intravitally. Like the glands, the ducts of the anterior and posterior pairs are also distinct in the texture and reaction of their content. The ducts of the anterior pairs of penetration glands lie medially to those of the posterior pairs.

The excretory system has 5 pairs of flame cells, 4 pairs in the body and the fifth in the tail. The excretory vesicle is situated near the posterior end of the body.

From its antero-lateral margins arise the main excretory tubes. The excretory tube of each side runs in an irregular wavy course up to the anterior level of the acetabulum and then turns posteriorly to divide, at about the level of the posterior margin of the ventral sucker, into the anterior and posterior collecting tubules. The backwardly directed portion of the excretory tube is slightly dilated at 2 points bearing cilia on the inner wall. The anterior collecting tubule gives rise to 2 capillaries each ending in a flame cell. The anterior flame cell is situated at about the level of the middle of the esophagus, while the posterior invariably lies laterally in the space between the 2 anterior pairs of penetration glands. The posterior collecting tubule gives rise to 2 capillaries; the anterior ending in a flame cell while the posterior divides further into 2 capillaries each terminating in a flame cell, 1 in the body and the other in the tail up to about one-eighth of the length of the tail stem. The flame cells draining into the posterior collecting tubule are situated at the level of the first and second third of the post-acetabular distance respectively. The excretory vesicle opens posteriorly into a wide and straight tube which extends through the tail to a little in front of the origin of the rami. It then divides into 2 branches which extend through the rami to open at their tips with slightly projecting dilatation. The arrangement and number of flame cells in the cercariae of *S. incognitum* may be represented by the formula: $2\ [2+2\ (+1)\ ] = 8\ (+2)$.

The nervous system consists mainly of 2 suboval large masses lying symmetrically a little behind the anterior quarter of the body length in the space between the bend of the ducts of the penetration glands and the body wall. The 2 nerve masses are connected by a nerve commissure passing dorsally over the gut.

Penetration into the Definitive Host

The cercariae of *S. incognitum*, like those of other schistosomes, attacks directly and penetrate the exposed skin and mucous surfaces. With the help of the piercing spines and lytic action of the secretion of the penetration glands the cercariae burrow in. The tail is left behind when the body penetrates.

An experiment was performed to find out the natural mode of infection. Three rats were given infection orally with water containing cercariae. Three other rats were given infection exclusively through the intact skin. To prevent the latter group of rats from acquiring oral infection through licking or drinking infected water the hind part of each rat was dipped into a narrow jar containing water infected with cercariae. The diameter of the jar was such that the rats could not turn in it. It was observed that by oral infection an average of 11 percent of 1,550 cercariae developed into adults and by cutaneous route of infection an average of 2 percent of 13,280 developed into adults.

Development in the Final Host

The cercariae of *S. incognitum* enter into the body of the final host through the mouth, skin, or mucous membranes, and develop into adult schistosomes in the hepatic, portal, and mesenteric veins in a period of 36 days.

Piglet No. 7 (male, 3 months old) was infected orally with the cercariae obtained from 3 snails. Some infected water was also smeared on its breast, abdomen, and groin. About 4,000 freshly shed cercariae were given to the animal over a period of 8 consecutive days. Thirty-six days after the first dose the feces was found to be positive for the characteristic eggs of *S. incognitum*. The eggs were viable and the

miracidia liberated when the feces were well diluted in water. The animal was sacrificed 3 months after the first appearance of eggs in its feces, and 50 normal adult specimens were recovered and identified as *S. incognitum*. Of the 50 specimens, 37 were male and 13 females of which 6 were in copula. The worms were recovered from different organs, e.g. 13 from liver, 18 from portal vein and 19 from mesenteric veins. The liver was slightly congested.

Piglet No. 11 (female 2 months old) was infected with 2,000 cercariae from 2 snails infected with miracidia obtained from the feces of piglet No. 7. The feces were found to be positive for the eggs of *S. incognitum* 5 weeks after the 1st infection. The animal was sacrificed and 36 normal specimens of *S. incognitum* were recovered from its different organs; 10 from liver, 17 from portal veins, 8 from mesenteric veins, and 1 from the pancreas. The liver was slightly congested.

DISCUSSION

The "shoulders" observed by Rao (1937) in the miracidium inside the egg shell were not traceable. The posteriormost cilia of the anterior group of epithelial cells are quite prominent, and if these touch the anteriormost cilia of the 2nd group of epithelial cells, the triangle thus formed on each side of the larva might present the appearance of "shoulders."

The flame cells of the miracidium of *S. incognitum* were found to be active even in eggs inside the mammalian tissue. This supports the view of Maldonado and Acosta-Matienzo (1947) who contradict the opinion of Reisinger (1923) that the function of the excretory system is the regulation of the osmotic equilibrium. According to Reisinger, the flame cells of the unhatched miracidium of *S. haematobium* are not active in the urine—an isotonic medium—but become active in water.

Besides the normal intermediate host, *L. acuminata* also exerted appreciable attraction on the miracidia of *S. incognitum*, but an infection either artificial or natural was never detected in this species of snail. Possibly *L. acuminata* may serve as the intermediate host of the parasite under conditions necessitating its adaptation to a new snail host as Stunkard (1946) conjectured.

Of all the known cercariae of mammalian schistosomes, the cercaria of *S. incognitum* resembles most closely that of *S. spindalis* in its morphology and flame cell formula. Nevertheless, it can be distinguished from the latter by several distinctive features. The gut in the cercaria of *S. incognitum* is divided into distinct intestinal ceca and in that of *S. spindalis* it is pear-shaped (plate I, figs. 6 and 7). The anterior penetration glands in the cercaria of *S. incognitum* are boot-shaped, while in the other they are pyriform. The terminal dilated portion in the excretory tubule in the ramus of the cercaria of *S. incognitum* is not marked off by constriction as is the case in the cercaria of *S. spindalis* (plate I, figs. 8 and 9). The flame cells in the cercaria of *S. incognitum* are situated more posteriorly than in *S. spindalis*, with the result that the flame cells in the tail lie at the anterior one-eighth and one-tenth of the length of the tail stem in the 2 cercariae respectively. The 2 can be further separated by the fact that the cercaria of *S. incognitum* remains in the top layer of water while the other is uniformly distributed at all levels. All other schistosome cercariae which rest near the surface, e.g. *S. japonicum* (Faust and Meleney, 1924), *Schistosomatium douthitti* (Price, 1931), *Bilharziella polonica* and *Schistosomatium pathlocopticum* (Porter, 1938), remain suspended with their

ventral sucker touching the surface film of water, but the cercariae of *S. incognitum* hang in the water with their furci touching the surface.

The pigs are such natural hosts that in fairly heavy infections there was no considerable pathological change except slight congestion.

SUMMARY

The detailed life cycle of *Schistosoma incognitum*, a common blood fluke of pig in India, has been worked out. The development of the embryo and hatching of the egg have been observed. The movements and morphology of the miracidium and its penetration of the snail host have been thoroughly studied. The larval development within the molluscan host has been elucidated. The movements and morphology of the cercaria have also been thoroughly studied. Penetration of the cercaria into the definitive host and its development into adult have been shown.

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Explanation of Plates

Schistosoma incognitum Chandler, 1926

Abbreviations: ap, apical papilla; at, anterior collecting tubule; c, ciliated area; cd, collecting duct; cet, caudal excretory tube; cg, cephalic gland; ct, collecting tubule; dgc, degenerate germ cell; ds, duct spine; ep, excretory pore; ep1, first row of epithelial cells; ep2, second row of epithelial cells; ep3, third row of epithelial cells; ep4, fourth row of epithelial cells; ev, excretory vesicle; f, furcal ramus; fc, flame cell; g, gut; gc, germ cell; hg, head gland; ho, head organ; ic, island of Cort; ld, lateral gland duct; lg, lateral gland; lp, lateral papilla; m, mouth; mt, main excretory tubule; n, nerve centre; nc, nerve commissure; oe, esophagus; pa, anterior penetration gland; pp, posterior penetration gland; pt, posterior collecting tubule; ts, tail stem; vs, ventral sucker.

Plate I

Figure 1. Dorsal view of the miracidium.
Figure 2. Miracidium showing the epithelial cells.
Figure 3. Ventral view of the miracidium showing the excretory system.
Figure 4. Lateral view of the cercaria.
Figure 5. A position of the furcal rami showing excretory pores.
Figure 6. The intestinal ceca.
Figure 7. Intestinal ceca of *S. spindalis.*
Figure 8. Terminal portion of the excretory tubule in the furcus.
Figure 9. Terminal portion of the excretory tubule in the furcus of *S. spindalis.*
Figure 10. Cercariae in the top layer of water

Plate II

Figure 1. Mother sporocysts; 5-day old infection.
Figure 2. Daughter sporocyst; 7-day old infection.
Figure 3. Daughter sporocyst; 14-day old infection.
Figure 4. Portion of an old daughter sporocyst showing germ balls; 18-day old infection.
Figure 5. Portion of the 22-day old daughter sporocyst showing fully formed cercariae, one of which is emerging through the birth pore.
Figure 6. Immature cercaria dissected out of the sporocyst.
Figure 7. The complete cercaria.
Figure 8. The body of the cercaria showing all the internal organs.

PLATE I

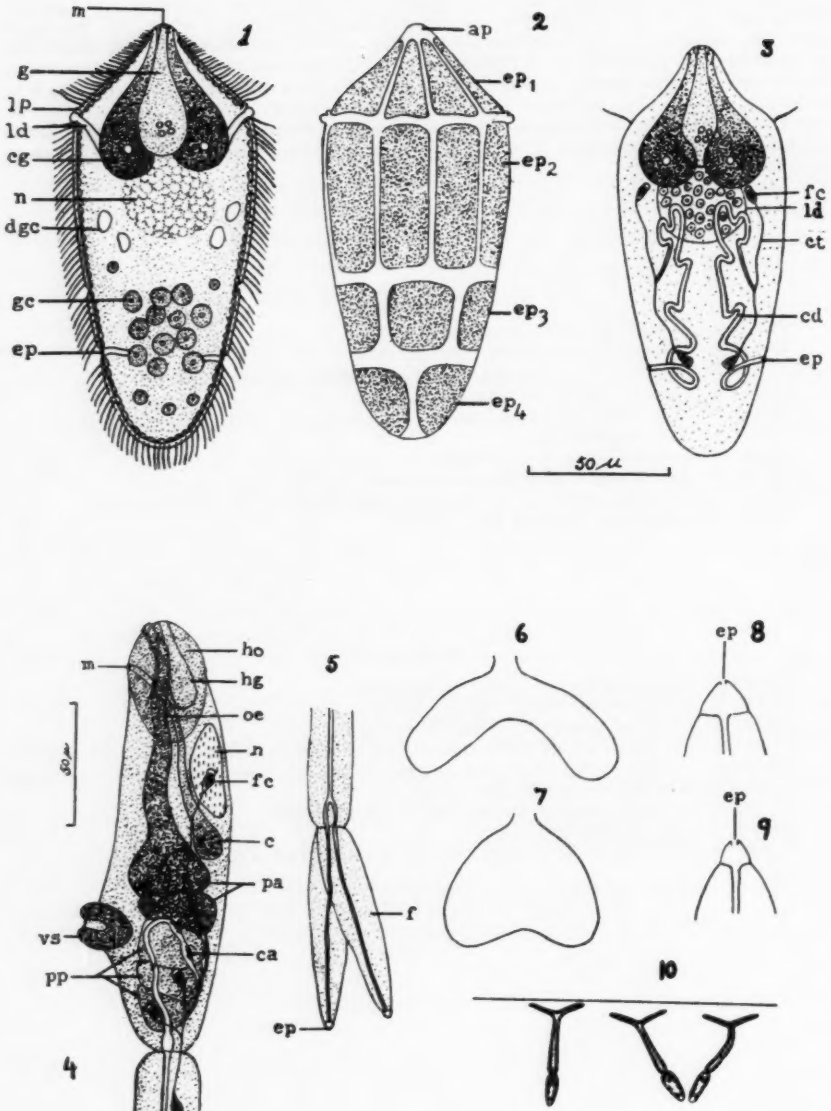
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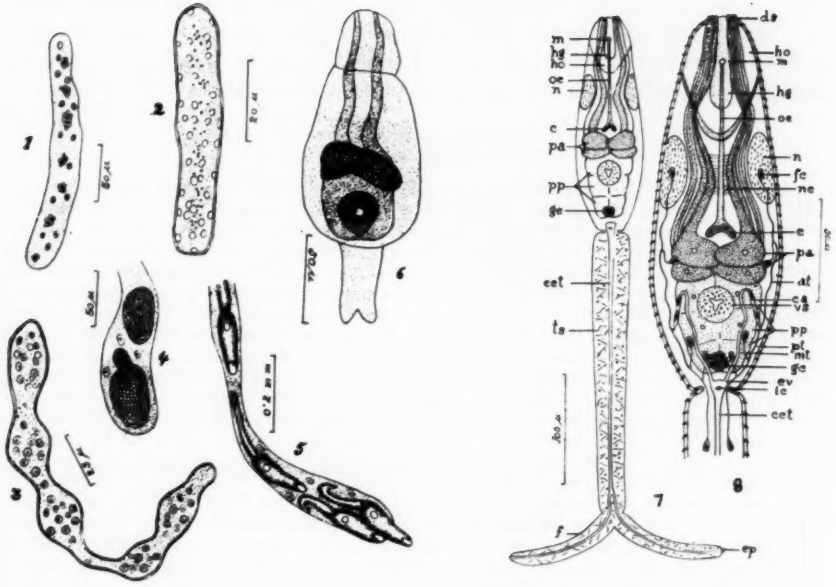
PLATE II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APORCHIS CONTINUUS N. SP. (TREMATODA: ECHINOSTOMATIDAE)*

JAMES E. MCCAULEY AND IVAN PRATT
Oregon State College, Corvallis**

The species described below was collected by Dr. Stuart E. Knapp of the Department of Veterinary Medicine at Oregon State College from the intestine of a short-billed gull, *Larus canus* Linnaeus, taken from Sauvies Island in the Columbia River north of Portland, Oregon. The material contained 8 echinostome trematodes which appeared to be an undescribed species of *Aporchis*. The name *Aporchis continuus* n. sp., refers to the uninterrupted row of marginal spines on the head collar.

The following description is based on all 8 specimens which were mounted entire except 1 which was serially sectioned. All measurements are in millimeters and are given first for the holotype and then as the range of all specimens in parentheses.

Aporchis continuus n. sp.
(Figs. 1–4)

Description: With characteristics of the genus. Body very long and filamentous, 22.0 (14.7 to 32.0) by 0.75 (0.60 to 0.97) wide at level of anterior margin of ovary. Body widest in posterior third, tapering gradually anteriorly to a long filamentous neck. Oral sucker at anterior extremity, 0.08 (0.08 to 0.15) in diameter. Ventral sucker about 2 times its diameter from the anterior end, 0.28 (0.28 to 0.35) in diameter. Genital pore at anterior margin of ventral sucker. Excretory pore at posterior extremity. Head collar notched ventrally. Spines not apparent on holotype, but paratypes show a single marginal, continuous row of about 48 spines measuring about 0.008 by 0.010 and 12 to 18 large spines in the notch which measure about 0.041 by 0.015 and overlie the pharynx in 2 groups. Cuticular spines on the surface of the neck and body are "segmentally" arranged to give the superficial appearance of a small cestode (fig. 4). Pharynx globular, 0.10 (0.08 to 0.12) in diameter. Short prepharynx and esophagus present. Intestinal crura reach nearly to posterior extremity. Testes tandem, ovoid, with greater axes longitudinal, close together near the posterior end (usually in the posterior 8th). Anterior testis 0.63 by 0.39 (0.63 to 0.80 by 0.20 to 0.48). Posterior testis 0.80 by 0.38 (0.75 to 0.85 by 0.20 to 0.50). Cirrus pouch slender and sinuous, extends 1.4 (1.2 to 2.0) behind ventral sucker, contains cylindrical seminal vesicle which measures 0.80 (0.55 to 0.80) long by 0.10 (0.10 to 0.12) in diameter. Ovary spherical, 0.27 (0.21 to 0.27) in diameter, close in front of anterior testis. Uterus spirally coiled, without descending arm. Egg-filled portion occupies space between anterior testis and mid-level of body. Vitellaria a row of follicles on either side of uterus, but extending only slightly into the middle third of the body length. Eggs 0.092 to 0.104 by 0.031 to 0.041, with filament at one pole about 0.46 long (fig. 3).

Host:—Larus canus Linnaeus, the short-billed gull, intestine.

DISCUSSION

Timon-David (1955) lists the following species in the genus *Aporchis: A. croaticus* Stossich, 1905, *A. segmentatus* Fuhrmann, 1915, *A. rugosus* Linton, 1927, *A. liouvillei* Dollfus, 1951, and *A. massiliensis* Timon-David, 1955. *A. continuus* differs from *A. liouvillei, A. rugosus,* and *A. segmentatus* in having the long axes

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of the testes longitudinal instead of transverse. It differs from *A. croaticus* in having a much shorter cirrus pouch and the vitellaria do not reach the cirrus pouch. *A. continuus* is close to *A. massiliensis* but lacks the lateral interruptions in the row of marginal spines on the head crown, the notch spines are longer, and the marginal ones are uniform in size and arrangement. Also the eggs are narrower and have shorter filaments than in *A. massiliensis*. The marginal spines are not visible on the holotype but do show on paratype No. 1.

The holotype and one paratype are deposited in the United States National Museum (Helm. Coll. No. 39252).

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Explanation of Plate I

Aporchis continuus n. sp.

Figure 1. Camera lucida drawing of holotype.

Figure 2. Diagrammatic drawing of anterior portion of worm showing a reconstruction of the head collar spines from the paratypes.

Figure 3. Camera lucida drawing of an ovum with its polar filament.

Figure 4. Camera lucida drawing of the anterior end of one of the paratypes.

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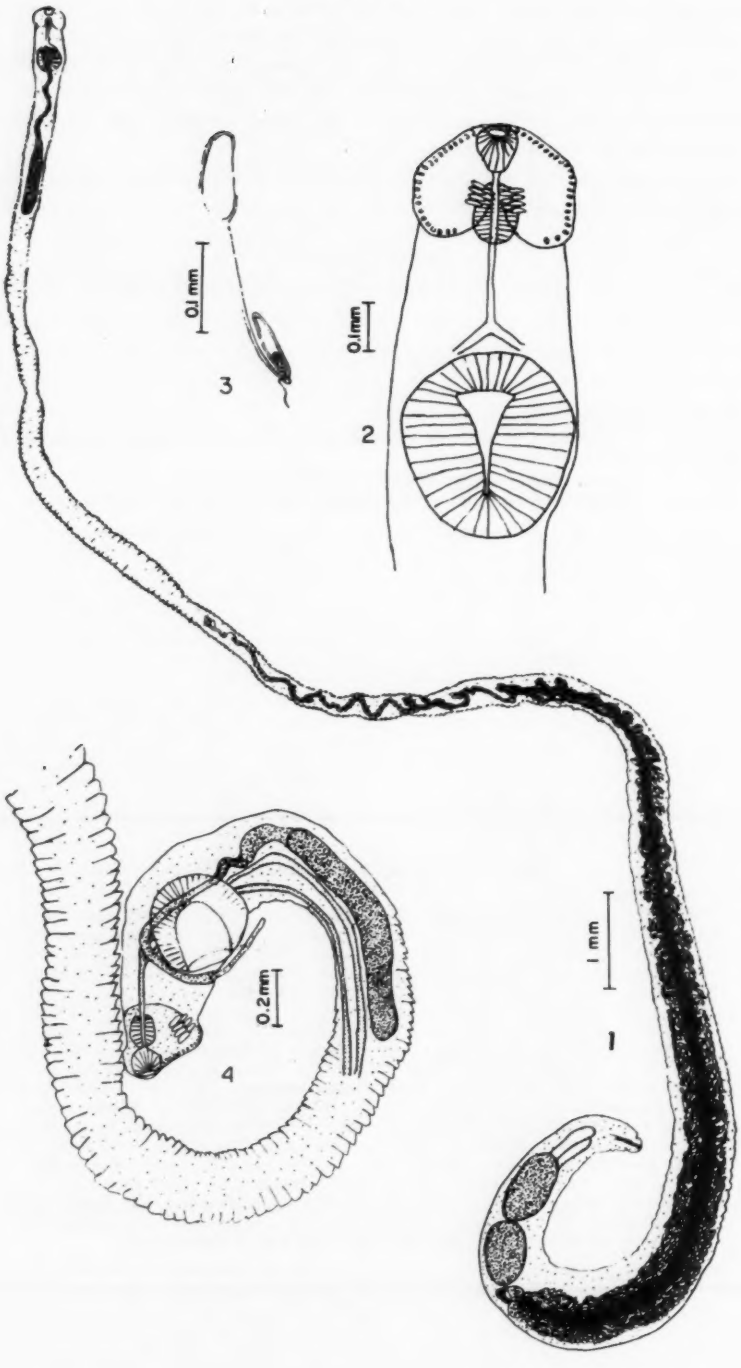
PLATE I



OBSERVATIONS ON THERAPEUTIC AND PROPHYLACTIC EFFECTS BY HOMOLOGOUS IMMUNE BLOOD AGAINST *SCHISTOSOMA MANSONI* IN RHESUS MONKEYS

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Although attempts to passively immunize mice against *Schistosoma mansoni* by the use of heterologous immune serum (from rats) have been unsuccessful (Stirewalt and Evans, 1953), it seems worthwhile to record the following brief efforts to induce immunity against mature and immature worms in rhesus monkeys by using homologous humoral substances. Parenthetically, it may be noted that conflicting results have been attained in attempts to confer passive immunity against *S. japonicum*. (For a review of this literature see Sadun and Lin, 1959.)

Five monkeys were used in the present work. One served as the donor; the remaining four were used as two pairs of test subjects in which one member of the pair was treated and the other served as a parallel untreated control. The two pairs were used, respectively, for observations on the effects of passive immunization attempts against mature and immature worms.

The pertinent features of the donor monkey, viz, the history of its infection and evidence that it had antischistosomal antibodies, are indicated by the following statements. The donor was a healthy male weighing 2.3 kg when infected percutaneously with 1000 *S. mansoni* cercariae; extensive experience in these laboratories has indicated such exposure induces infection with 200 to 250 mature worms. Egg production, as reflected by fecal examinations, reached a peak of 1250 eggs per ml of fecal material 2.5 months after exposure, gradually declined during the next 15 months, but continued at very low levels until the animal was sacrificed (final weight 11.2 kg) 32.5 months after exposure. Perfusion and search of the liver and portal-mesenteric system yielded a single pair of living worms. Assays of circumoval precipitins (as performed by Oliver-Gonzalez et al, 1955) and of pericercarial membrane reactions (as performed by Kagan, 1955) were conducted on several serum samples between 4.5 and 30 months after exposure. All samples yielded highly positive reactions; precipitins formed around approximately 45 percent of the viable eggs and pericercarial membrane reactions occurred at serum dilutions of 1:128. These findings are construed as evidence of a relatively high level of antibodies in the donor during the critical phases of the transfusion experiments.

Heparinized whole blood was drawn from a peripheral vein of the donor and injected immediately intravenously into the recipients. The first few ml were given slowly so as to enable recognition of transfusion reactions; consistent with extensive transfusion work on monkeys in these laboratories, no reactions were seen. It was then considered that transfusing whole blood would enable the most effective use of the available humoral substances with minimal manipulations.

Table I depicts the experiment to see whether immune blood would exert an effect against mature worms sufficiently deleterious to influence egg counts (by a modification of the acid-ether procedures of Telemann, 1908), with due allowance

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TABLE I. *Attempt to immunize against mature worms.*

Monkey		Days After Percutaneous Exposure to 500 *S. mansoni* Cercariae											
		35	40	42	44	47	49	51	56	61	68	70	75
G221, treated (2.3 kg ♀)	MI blood*			4	15	14	15	14					
	Eggs/ml feces	0	160	140	245	595		800	825	1200	730	1050	2050
G216, control (2.15 kg ♀)	Eggs/ml feces	20	30	170	260	725		700	500	470	380	1280	570

* From a donor during the 27th month of infection.

for the time requisite for eggs to appear or disappear from the feces. Even though a total of 62 ml of blood from a donor during its 27th month of infection was transferred into a 2.3 kg monkey, no effect on egg production in the recipient relative either to the parallel control or to the usual findings in a large number of other monkeys was induced.

Table II summarizes the experiment in which transfusions were applied against

TABLE II. *Attempt to immunize against immature worms.*

Monkey		Days After Percutaneous Exposure to 1000 *S. mansoni* Cercariae								
		0	1	3	6	8	10	42	44	49
6V, treated (4.65 kg ♀)	MI blood*	22	15	20	20	16	20			
	Eggs/ml feces							0	40**	550
7V, control (4.95 kg ♀)	Eggs/ml feces							0	20**	430

* From a donor during the 30th month of its infection.
** Hatching procedures were done and eggs were shown to be viable.

immature worms. The time required for eggs to appear in the feces, egg counts, and egg hatchings were used as criteria for assaying effect. By inspection of the data, it will be seen that although a total of 113 ml of blood from the donor during the 30th month of its infection was given, no differences with respect to any of these criteria occurred between the recipient and its parallel control. Moreover, the infections in both animals conformed to the pattern repeatedly observed by us in a large number of monkeys.

In conclusion, it is to be noted that relatively massive transfusions with immune blood, corresponding to approximately one-third of the blood volume of the recipients, failed to exert effect against either mature or immature worms on the basis of the criteria employed. It is recognized that these attempts do not conclusively prove that passive immunization against *S. mansoni* is impossible. Possibly the use of hyperimmune serum, a large number of animals, other types of hosts, or other criteria might yield evidence of some effect. Nevertheless, the present study raises serious doubt as to the practical efficacy of passive immunization against *S. mansoni* and is being recorded with the hopes that it may stimulate others to report their observations on this general subject.

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RESEARCH NOTES

TWO PARASITES FROM COATI IN SOUTHERN ARIZONA.

On April 14, 1956, I took an old male coati, *Nasua narica*, on the Peterson Ranch in the Huachuca Mountains. Two species of parasites were collected from this old male. One species of ectoparasite, *Pulex irritans* (Linne), was abundant on this specimen. A nematode, *Physaloptera rara* (U.S.N.M. Helm. Coll. No. 38067), was found in the stomach. As far as could be determined from the literature, this parasite is being reported for the first time from the coati. I wish to thank Dr. C. Andresen Hubbard of Tigard, Oregon, for identifying the fleas and May-Belle Chitwood, Agriculture Research Center, U. S. Dept. of Agriculture for identifying the nematode.—BOBBY J. NEAL, *Division of Natural Resources, Humboldt State College, Arcata, Calif.*

THE INFLUENCE OF SEITZ FILTRATION ON THE ACTIVITY OF *SCHISTOSOMA MANSONI* (ADULT WORM) ANTIGEN.

Several investigators recommend the filtration, through sterilizing Seitz sheets, of schistosome antigens to be used for intradermal tests (Mayer and Pifano, 1945, Rev. San. y Asist. Soc. **10:** 3; Pessôa and Barros, 1953, Hospital, Rio de Janeiro **43:** 19, for *S. mansoni* extracts; Alves and Blair, 1946, Lancet **251:** 556, for *S. haematobium* extracts; and Pesigan et al, 1954, J. Phil-M. A. **30:** 14, for *S. japonicum* antigens). Nevertheless, the influence of Seitz filtration on the skin test activity of such antigens was not investigated.

Adult worm (*S. mansoni*) extract, 1:1,000 by weight in Coca-merthiolate solution, was prepared as described elsewhere (Pellegrino, Brener and Silva, 1958, Rev. Brasil. Malariol. **10:** 291) and a sample of the same extract was filtered through EKS sterilizing Seitz sheet. Nitrogen determination showed a marked decrease of total N from 0.048 mg per ml before filtration to 0.004 mg per ml after Seitz filtration. Practically all the filtrate nitrogen consisted of non-protein N.

Samples (0.05 ml) of non-filtered and Seitz-filtered antigens were intradermally injected on the volar surface of the forearm of 21 adult patients with active schistosomiasis mansoni. After 15 minutes the outline of the wheals was drawn in ink and its area determined following the method of Pellegrino and Macedo (1956, Rev. Brasil. Malariol. **8:** 499). A mean area of 2.19 ± 0.49 sq cm was found for the non-filtered extract and of 0.86 ± 0.27 sq cm after Seitz filtration.

Taking the wheal area of 1.2 sq cm as the minimum area for a positive test (Pellegrino, 1958, Bull. World Health Org., **18:** 945), the skin test was positive in all patients when non-filtered extract was used and in only one patient with the same extract after Seitz filtration.

The adsorptive activity of Seitz filter is well known (Wilke, 1956, Pharm. Ind., **18:** 428) and our data show that a great amount of antigenic material of *S. mansoni* extract is retained in EKS sterilizing sheet. When, besides the use of preservatives as 0.4 percent phenol or 1:5,000 merthiolate, a better sterilizing effect is desired, the extracts can be heated to 56 C for 1 hour on 3 consecutive days as suggested by Pesigan et al (1951, J. Phil. Med. As., **27:** 212). This procedure does not significantly change the skin test activity of the extracts.—J. PELLEGRINO AND BENEDITO A. RODRIGUES, *Instituto Nacional de Endemias Rurais, Centro de Pesquisas de Belo Horizonte, Brasil.*

HAWAIIAN HELMINTHS. IV. *PARACARDICOLA HAWAIENSIS* N. GEN., N. SP. (TREMATODA: SANGUINICOLIDAE) FROM THE BALLOON FISH, *TETRAODON HISPIDUS* L.*

W. E. MARTIN

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The fish blood flukes described here were collected from the mesenteric veins of *Tetraodon hispidus* at the Hawaii Marine Laboratory, University of Hawaii. The fish were collected by trapping in Kaneohe Bay, Oahu. All of the 10 fish examined for blood flukes were found to harbor the eggs or the eggs and *Paracardicola hawaiensis*. The presence of eggs was determined by examining a small piece of host liver flattened between 2 microscope slides. Hundreds of eggs were found in most of the 10 hosts.

The worms, secured by flushing out mesenteric veins, were fixed in cold Heidenhain's solution and stained with celestine blue B. They were mounted in H.S.R. Microscopic Mounting Medium.

All measurements are in millimeters.

Paracardicola n. gen.

Generic diagnosis: Sanguinicolidae. Body small, slender, laterally spined along most of length. Oral sucker rudimentary. Esophagus long, about one-third of body length, slender, slightly sinuous. Intestine H-shaped, posterior ceca considerably longer than anterior. Gonads in posterior half of body, anterior testis immediately in front of ovary, posterior testis behind posterior extent of uterus. Seminal vesicle elongate and saccular. Ovary compact. Uterus postovarian, relatively long, with few loops. Small vitelline glands extend from mid-esophageal level to ovary. Eggs with terminal spine. Genital pores separate but close together, near mid-seminal vesicle level. Parasitic in circulatory system of marine fish.

Genotype: P. hawaiensis in *Tetraodon hispidus* L.

Paracardicola hawaiensis n. sp.

Species diagnosis: Description based on 10 specimens. With characters of genus. Body elongate, slender, laterally spined, 0.72 to 1.92, av. 1.26 in length and 0.04 to 0.11, av. 0.079 in maximum width. Body with lateral, comb-like spines along most of length. Oral sucker terminal, weakly developed, approximately 0.016 in diameter. Esophagus tubular, elongate, 0.24 to 0.57, av. 0.46 in length and 0.006 to 0.012, av. 0.011 in maximum width. Intestine H-shaped, anterior ceca shorter than posterior. Anterior ceca of approximately the same length, 0.037 to 0.118, av. 0.068 long and 0.015 to 0.025, av. 0.021 in maximum width. One posterior cecum longer than the other, the shorter cecum 0.09 to 0.48, av. 0.29 and the longer 0.11 to 0.53, av. 0.32 in length. Maximum widths of posterior ceca similar, 0.015 to 0.025, av. 0.021. Anterior testis immediately in front of ovary, 0.075 to 0.3, av. 0.17 in length and 0.03 to 0.081, av. 0.06 in maximum width. Posterior testis behind uterus, not as well developed as anterior testis, 0.087 to 0.23, av. 0.16 in length and 0.028 to 0.06, av. 0.04 in maximum width. Ducts from testes enter anterior end of seminal vesicle. Seminal vesicle elongate, saccular, weakly convoluted, sperm-filled, 0.12 to 0.15, av. 0.137 in length and approximately 0.025 in maximum width. The seminal vesicle bends anteriad for a short distance to terminate as the male genital pore. Although the wall of the terminal portion of the male duct is slightly thickened, there is no distinct cirrus. The

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Contribution No. 127, Hawaii Marine Laboratory, University of Hawaii, Honolulu.

compact ovary is immediately posterior to the anterior testis. The oviduct is joined by the vitelline duct a short distance posterior to the ovary. The small vitelline glands extend posteriorly from the mid-esophageal level to the posterior margin of the ovary. They are lateral to and between the intestinal ceca. The uterus extends posterior to the seminal vesicle, then loops anteriad to a level near the ovary, then bends posteriorly to open at the female genital pore. The distal portion of the uterus is expanded and has thickened walls. It probably is here that the egg shell is formed. There is a raised group of cells surrounding the female pore. Eggs about 0.08 long and 0.037 wide with terminal spine approximately 0.009 long. These eggs were recovered from the host's liver and contained a ciliated miracidium lacking eyespots. A tubular excretory bladder opens at the posterior end of the body.

Type specimen: *Paracardicola hawaiensis*, deposited as number *5716* in the Hancock Parasitology Collection.

Host: *Tetraodon hispidus* L., balloon fish, in mesenteric veins.

Locality: Kaneohe Bay, Oahu, Hawaii.

DISCUSSION

Paracardicola most closely resembles the genus *Cardicola* which Short (1953) erected with *Psettarium cardiocolum* Manter, 1947, as type and to which he assigned *C. laruei*. Short changed the spelling of *cardiocolum* to *cardicola*. The correct spelling of Manter's species is *Cardicola cardiocolus* following the Copenhagen Decisions on Zoological Nomenclature (1953) which states, "names consisting of words ending with the Latin term—'cola' are to be treated as masculine, unless the name is taken from a word given in the standard Latin dictionary as being of common gender and the author of the name stated in the original description that the name was feminine in gender." *Paracardicola* differs from *Cardicola* and all other genera of fish blood flukes in having 2 testes. *Cardicola* and *Selachohemecus* Short, 1954, have 1 testis and the other genera have multiple testes (see Short, 1954). In *Paracardicola* the posterior intestinal ceca reach the anterior portion of the preovarian testis while in *Cardicola* they reach the ovary. There are additional differences in the gonoducts.

As in *Cardicola cardiocolus* (Manter, 1947), the uterine eggs appear to be without shells. The shell may be laid down in the lower end of the uterus and the egg laid soon afterward. Eggs of *Paracardicola hawaiensis* are very numerous in the liver of *Tetraodon hispidus* and must do considerable damage to this organ.

Manter (1947) and Short (1953) placed their new species of fish blood flukes in the family Aporocotylidae, but Yamaguti (1958) places both of these species in the Sanguinicolidae reserving Aporocotylidae for a single genus, *Aporocotyle*.

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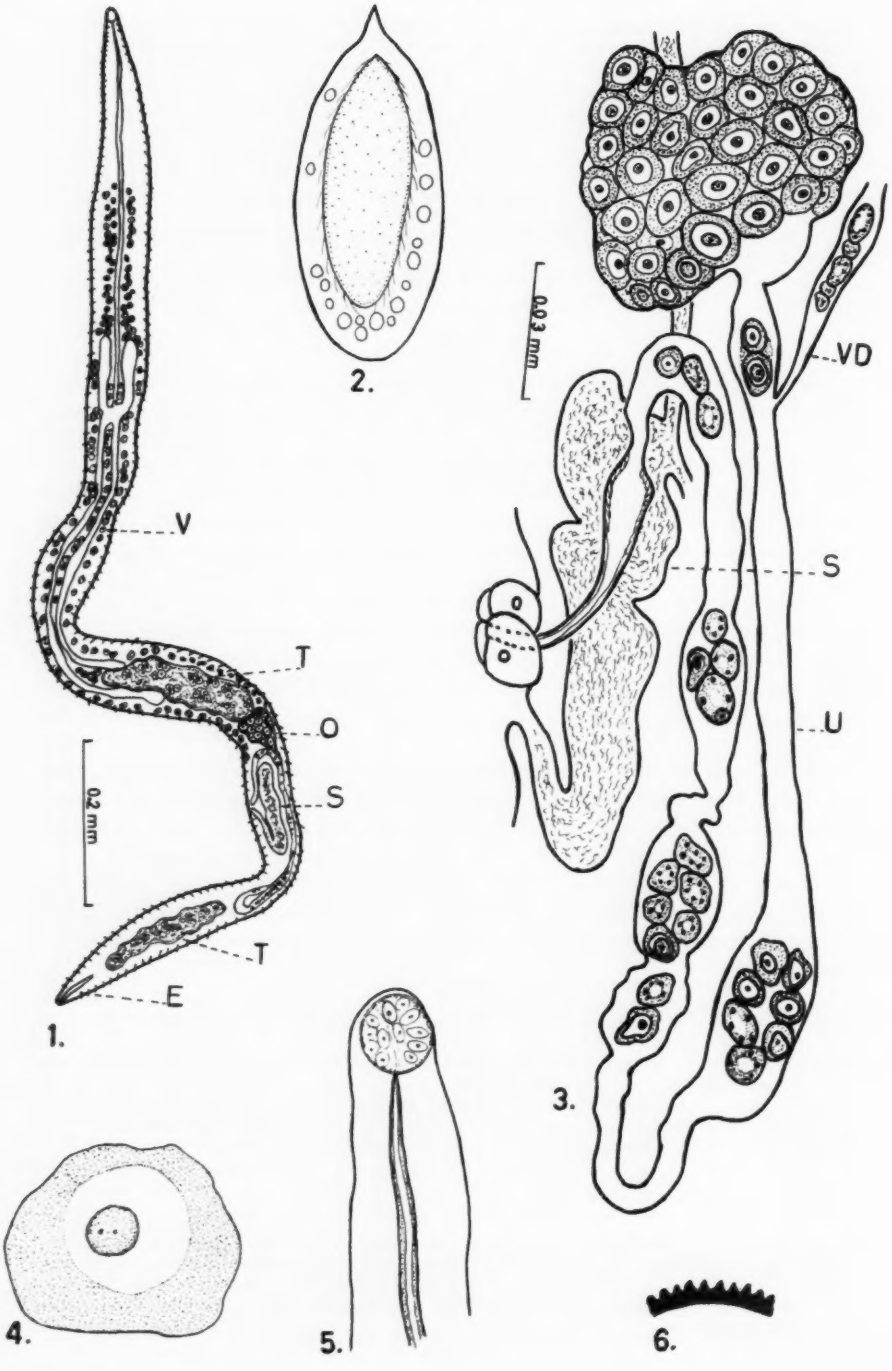
EXPLANATION OF PLATE I

FIGURES 1–6. *Paracardicola hawaiensis:* 1, Entire worm; 2, shelled egg containing miracidium and globules; 3, ovary and main portions of genital ducts; 4, egg immediately after release from ovary; 5, anterior end of worm showing rudimentary sucker; 6, comb-like spine from side of body; E, excretory bladder; O, ovary; S, seminal vesicle; U, uterus; V, vitellaria; VD, vitelline duct.

PLATE I



SOME DIGENETIC TREMATODES OF EELS OF HAWAII*

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The following trematodes were collected by the junior author in 1949. The eel hosts were taken from a rotenone poison station in shallow water inside the reef of Hauula Park on the northeast coast of Oahu. Dr. William A. Gosline, University of Hawaii, conducted the poison station and identified the fishes collected.

Eight families and some 42 species of eels are known from Hawaii. The 64 specimens examined represented 4 families and 6 species and were distributed as follows: Congridae: *Conger cinereus,* 37 examined; Ophichthyidae: *Myrichthys maculosus,* 1; Moringuidae: *Moringua javanica,* 5; Muraenidae: *Gymnothorax buroensis,* 19; *G. ?flavomarginata,* 1; *G. steindachneri,* 1. Trematodes, recovered from about 75 percent of the hosts, were killed under a cover glass with F.A.A. fixative, stored in 70 percent alcohol, stained in Delafield's hematoxylin, and mounted in Permount.

Type specimens are deposited in the United States National Museum. All measurements are in millimeters unless otherwise stated.

Not included in this paper are 3 species of Hemiuridae described in another paper (Manter and Pritchard, 1960).

Family Bucephalidae

Dolichoenterum microtylum n.sp.
(Figs. 1–4)

Host: *Conger cinereus* Ruppell, conger eel, locally known as puhi uha (slippery eel) and puhi ula (red eel); 12 specimens (2 mature, 9 immature, and posterior half of a mature specimen) in 3 of 37 hosts examined.

Location: Intestine.

Holotype: U. S. Natl. Mus. Helm. Coll., No. 39156.

Description: Body long and slender; length 3.979 to 6.570 by 0.504 to 0.548 greatest width; all reproductive organs in posterior half of body which is wider and more cylindrical than the anterior half; both ends truncate; body spines numerous anteriorly, disappearing posteriorly. Anterior sucker cup-like, 0.168 to 0.219 wide by 0.146 to 0.153 long; dorsal side with 8 papillae. Mouth about 1/6 to 1/5 body length from anterior end, surrounded by a weakly developed but distinct oral sucker, 0.033 long by 0.025 deep; prepharynx 0.032 to 0.058 long; pharynx ovoid or pyriform, 0.136 to 0.153 long by 0.128 to 0.131 wide; esophagus somewhat shorter than prepharynx; cecum with short anterior appendix, very long, almost straight, extending past gonads to near posterior end of body.

Testes tandem, separated by ovary, subequal, 0.307 to 0.382 by 0.219 to 0.295 wide. Cirrus sac thick-walled except at each end where its wall thins, 0.496 to 0.548 by 0.167 to 0.212, overlapping posterior testis slightly. Seminal vesicle ovoid, in anterior third of cirrus sac; pars prostatica straight or nearly so; ejaculatory duct short, leading into hermaphroditic duct of genital papilla; genital atrium shallow, opening into terminal depression of body into which excretory pore also opens.

Ovary intertesticular, wider than long, 0.190 to 0.204 by 0.256 to 0.263; Mehlis' gland and Laurer's canal immediately postovarian; uterus coils anteriorly about 1 mm. then posteriorly; metraterm short, joining ejaculatory duct near base of genital papilla; eggs yellowish, thin-

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shelled, 20 to 23 by 12 to 17 microns; vitelline follicles rounded to ovoid, extending from anterior testis to near midbody, confluent anterior to anterior testis, 55 to 57 follicles in 2 specimens, 70 in another. Excretory vesicle extending to anterior end of cirrus sac.

Discussion: Four species of *Dolichoenterum* have been named: *D. longissimum* Ozaki, 1924, from conger eels of Japan and New Zealand; *D. lamirandi* Carrère, 1937, experimentally in *Hyla arborea; D. manteri* Tendeiro, 1954, from the conger eel, coast of Portugal; and *D. magnum* Yamaguti, 1959, from the conger eel of Japan. No figure of *D. lamirandi* was given but its description indicates an ovoid body, testes side by side, and intertesticular ovary. This body shape and arrangement of gonads is very different from *Dolichoenterum,* and the species probably belongs in another genus. We consider it a *species incertus.*

D. microcotylum differs from all other gasterostomes in possessing an oral sucker which, however, was not visible in immature specimens. Its occurrence supports the view that the pharynx-like structure of gasterostomes is truly a pharynx. The name microcotylum (minute sucker) refers to this oral sucker.

D. microcotylum differs from *D. longissimum* in more anterior mouth, more numerous and confluent vitelline follicles. *D. manteri* has only 5 anterior papillae, a shorter cecum reaching only slightly beyond midbody, and larger gonads closer together. *D. magnum* appears to differ chiefly from *D. manteri* only in having 8 anterior papillae. The 5 papillae of the single specimen of *D. manteri* were indistinct and 8 may prove to be present as in other species of the genus. Tendeiro has indicated (in correspondence) that *D. manteri* also has an inconspicuous oral sucker.

Family Opecoelidae

Opegaster ditrematis Yamaguti, 1942
(Fig. 5)

Hosts: *Moringua javanica* (Kaup), Java whip eel, 5 specimens from 5 hosts; *Myrichthys maculosus* (Cuvier), spotted snake eel, known locally as puhi laau, 7 specimens from 1 host.
Location: Intestine.

Discussion: The 12 specimens showed considerable variation. For example, the testes were smooth to slightly or deeply bilobed; the genital pore varied from midesophageal level to opposite base of the pharynx. The vitellaria usually began opposite posterior part of the esophagus but sometimes as far forward as base of the pharynx. In the former cases, transparent vitelline follicles free of yolk granules extend to level of the pharynx. The seminal vesicle may overlap the acetabulum slightly or may extend to its posterior edge. Egg size varied from 50 to 64 by 26 to 45 microns.

The 10 acetabular papillae, 5 on each margin of the acetabulum, are inconspicuous and invisible if the lips are pressed together. Yamaguti (1942) described "several" papillae on the anterior and posterior margin of the acetabulum, but his figure shows a total of 10, as in our specimens. He had only 1 specimen the characteristics of which agree with variations found in our material except that the Hawaiian specimens showed: (1) no vitelline follicles with yolk granules anterior to the genital pore; (2) the posterior testis is smooth or bilobed rather than with 2 small indentations on each end; (3) the sucker ratio is 1: 1.3 to 1.94 rather than 1: 2. These differences do not seem sufficient to warrant a new species, although the fish hosts are quite different. Only 1 other species of *Opegaster, O. lutianai* Bravo and Manter, 1959, has 10 acetabular papillae.

Helicometra dochmosorchis n.sp.
(Fig. 6)

Host: *Gymnothorax buroensis* Bleeker; 1 specimen from 19 hosts examined.
Location: Intestine.
Holotype: U. S. Natl. Mus. Helm. Coll., No. 39157.
Description: Body 2.238 by 0.804; greatest width at midbody; both ends tapered to a rounded point. Oral sucker round, 0.134 in diameter; acetabulum one-third body length from anterior end; 0.302 wide by 0.315 long, with transverse aperture; sucker ratio 1:2.3. Prepharynx 0.042 long; pharynx 0.060 long by 0.067 wide; esophagus muscular, 0.201 long; intestinal bifurcation just posterior to middle of forebody; ceca extending to near posterior end of body.

Testes unlobed but each has a slight, broad indentation laterally; oblique; contiguous, overlapping by about half length; 0.268 to 0.281 long by 0.194 to 0.235 wide. Posttesticular space 0.516. Cirrus sac claviform, overlapping anterior edge of acetabulum, posterior two-thirds straight, anterior one-third bent toward left, 0.496 long by 0.074 wide. Seminal vesicle loops backward a short distance near its middle; prostatic cells surround its narrowed distal half; cirrus short and weak. Genital pore submedian, slightly to left of middle of esophagus.

Ovary 3-lobed, anterior to right testis and to right of anterior half of left testis, contiguous with both testes. Mehlis' gland large, preovarian; seminal receptacle laterodorsal to ovary, 0.114 by 0.168; pore of Laurer's canal dorsal, to right of ovary and posterior to seminal vesicle. Vitelline follicles from posterior part of esophagus to posterior end of body; dorsal, ventral, and lateral to ceca, very nearly confluent posterior to testes and anterior to acetabulum. Uterus coiled between ovary and acetabulum, following cirrus sac to genital pore. Eggs with medium length filaments (2 to 3 times the egg length), collapsed, 42 to 46 by 24 microns. Excretory pore terminal; excretory vesicle tubular, extending forward to level of ovary.

Discussion: The name *dochmosorchis* is from *dochmos,* oblique, and *orchis,* testis. In its oblique testes this species resembles the type species, *H. pulchella* (Rud., 1819). All other species in the genus have tandem testes. It differs from *H. pulchella* in much larger sucker ratio, greater posttesticular space (practically lacking in *H. pulchella*), more anterior genital pore, smaller gonads, and smaller eggs.

Helicometrina quadrorchis n.sp.
(Figs. 7–8)

Hosts: *Gymnothorax buroensis* Bleeker; 12 specimens from 5 of 19 hosts.
Gymnothorax steindachneri Jordan & Evermann, 1 specimen from 1 host.
Location: Intestine.
Holotype: U. S. Natl. Mus. Helm. Coll., No. 39159.
Description (based on 13 specimens): Body plump, tapered anteriorly, truncate at posterior end; 0.623 to 2.365 long by 0.288 to 0.771 wide; widest at acetabular level. Oral sucker 0.080 to 0.288 wide by 0.074 to 0.181 long. Acetabulum one-third distance from anterior end; 0.118 to 0.402 wide by 0.134 to 0.422 long; aperture flattened on either anterior or posterior edge. Sucker ratio 1:1.3 to 1.8. Prepharynx very short or lacking; pharynx 0.040 to 0.075 long by 0.047 to 0.107 wide; esophagus muscular, 0.047 to 0.168 long; bifurcation posterior to middle of forebody; ceca ending lateral to posterior testes or posterior to them.

Testes 4, intercecal, in posterior third of body, arranged more or less in tandem pairs, shapes varying from rounded to indented to lobate, posterior testes usually larger; posttesticular space from almost none (Fig. 8) to 1/6 body length (Fig. 7). Cirrus sac clavate, overlapping acetabulum 1/3 to 3/4 length of that organ; seminal vesicle widest at base of cirrus sac, extending straight forward to past middle of cirrus sac, looping backward, then turning anteriorly, narrowing gradually; scattered prostatic cells around distal third of seminal vesicle; cirrus weak. Genital pore median or submedian at level of esophagus.

Ovary deeply trilobed; sometimes the lobes are subdivided; median to submedian, ventral to anterior testes; Mehlis' gland large, diffuse, preovarian; seminal receptacle large, preovarian, to right or left, 0.082 to 0.134 by 0.094 to 0.201; Laurer's canal conspicuous, with several coils, pore dorsal, lateral to ovary on side opposite from seminal receptacle. Vitellaria begin at midesophageal or at bifurcation level and extend to posterior end of body; confluent anterior to acetabulum but not at posterior end of body. Eggs 40 to 53 by 20 to 32 microns, with long filament. Excretory pore terminal; excretory vesicle extending forward to posterior testes, sometimes between them almost to ovary.

Discussion: This species is the only one in the genus to possess 4 testes; other species have 5, 7, or 9. It is much like *Helicometra dochmosorchis* which, however, has only 2 testes. The deeply bilobed condition of the testes in some specimens of *H. dochmosorchis* suggests how 4 testes may have arisen. Manter (1933) has noted that *Helicometrina parva* was very similar to *Helicometra execta* except for the increased number of testes.

Family Acanthocolpidae

Acaenodera placophora n.gen., n. sp.
(Figs. 9–16)

Host: *Conger cinereus* Ruppell, conger eel; 10 specimens in 14 hosts.
Location: Intestine.
Holotype: U. S. Natl. Mus. Helm. Coll., No. 39155.
Description (based on 5 specimens): Body elongate; sides nearly parallel except for tapered prepharyngeal region; posterior end rounded; 2.370 to 3.351 long by 0.0438 to 0.533 wide. Eyespot pigment on either side of prepharynx midway between oral sucker and pharynx. Three distinct sets of spines present: (1) small, sharp body spines decreasing in both size and number from a level anterior to pharynx to level of ovary; (2) 2 lateral groups of 12 to 18 conspicuously enlarged, recurved spines extending from just posterior to oral sucker to level of eyespots, decreasing in size to merge with body spines (Fig. 12); (3) 17 flat plates in 7 midventral rows (2-3-2-3-2-3-2) beginning just posterior to oral sucker and decreasing in size to level of eyespots (Fig. 11). No peribuccal spines. Spines, particularly the large ones, may be lost in macerated specimens; lateral spines may leave basal stumps only. Oral sucker terminal; mouth directed anteriorly; oral sucker 0.094 to 0.119 wide by 0.060 to 0.073 long. Acetabulum about one-third body length from anterior end, 0.190 to 0.241 wide by 0.180 to 0.238 long; sucker ratio 1 : 2 to 2.4.

Prepharynx 0.40 to 0.55 long (0.298 in contracted state); pharynx 0.095 to 0.146 long by 0.088 to 0.121 wide; esophagus short and broad; ceca ending in ani (3 specimens) or a shallow uroproct, depending on degree of body contraction.

Testes tandem, contiguous or very close together; anterior testis 0.289 to 0.380 long by 0.162 to 0.241 wide; posterior testis generally tapered posteriorly, 0.316 to 0.496 long by 0.170 to 0.212 wide. Genital pore median, immediately preacetabular. Cirrus sac clavate, curving around edge of acetabulum, extending one-fourth to a little over one-third distance from acetabulum to ovary, containing a saccular seminal vesicle, thin-walled cirrus, and prostatic cells. Seminal vesicle an elongate sac, one-third to one-half length of cirrus sac; cirrus with hair-like spines except for basal enlarged portion.

Ovary median, immediately pretesticular, rounded; 0.133 to 0.161 long by 0.124 by 0.175 wide. Uterus preovarian; metraterm begins opposite acetabulum, glandular, thin-walled, about 0.13 long. Genital atrium tubular, about 0.13 long by 0.05 wide, thin-walled. Laurer's canal well developed, pore dorsal to ovary; canal thin-walled, distal portion curves to enter a small spherical cellular portion from which a narrow tube first enlarges, then curves to enter ootype. Seminal receptacle lacking; sperm cells in proximal portion of uterus. Vitellaria from near base of seminal vesicle to posterior end of body, circumcecal, confluent near posterior end of body. Eggs 60 to 74 by 46 to 61 microns. Excretory pore terminal when ani are present; otherwise opening into a shallow uroproct; excretory vesicle not traced.

Generic Diagnosis of Acaenodera: Acanthocolpidae. Body spines of 3 types: usual body spines, large lateral spines opposite prepharynx, and short rows of plate-like spines ventral opposite anterior half of prepharynx; peribuccal spines absent; eyespots present; long prepharynx, short esophagus; ani or uroproct present; genital pore immediately preacetabular; gonads tandem, close together, in posterior half of hindbody; cirrus sac extending posterior to acetabulum; cirrus with hair-like spines; short, tubular genital atrium; uterus entirely preovarian; seminal receptacle absent; eggs large, relatively wide, thin-shelled; vitelline follicles extensive from near base of cirrus sac to posterior end of body. Like *Stephanostomum* except for lack of peribuccal spines and presence of ventral plates and large lateral spines in neck region. Resemblance to *Deropristis* probably coincidental. Type species: *A. placophora* from *Conger cinereus* Ruppell, Hawaii. The name, *Acaenodera,* from *akaina,* spine and *dera,* neck, refers to the striking spination of the forebody.

Discussion: The heavy lateral spines and still heavier ventral plate-like spines of this genus give it a superficial resemblance to *Deropristis* Odhner, 1902, in which,

however, the median spines are dorsal. One species of *Deropristis* occurs in *Anguilla*. The lack of a seminal receptacle; the preovarian uterus; relatively few, large eggs; and extensive vitellaria reaching to the posterior end of the body are characters which place *Acaenodera* in the Acanthocolpidae rather than in the Depropristinae (a subfamily placed with some uncertainty in the Lepocreadiidae by Cable, 1952). It is related to *Stephanostomum* and *Tormopsolus* but differs from both in its remarkable spination.

Modification of body spines appears to have undergone parallel development in genera of Depropristinae and of Acanthocolpidae, as shown in the following comparisons:

Spination	Depropristinae (Lepocreadiidae?) genera	Acanthocolpid genera
Oral sucker unarmed; no unusual spination	*Skrjabinopsolus* Ivanov, 1935 (Syn. *Pristotrema* Cable, 1952)	*Tormopsolus* Poche, 1926
Oral sucker unarmed; enlarged body spines present	*Deropristis* Odhner, 1902	*Acaenodera*
Oral sucker with large spines; body spines not enlarged	*Pristicola* Cable, 1952	*Stephanostomum* Looss, 1899

A difficulty in dealing with acanthocolpids is that the larger spines are rather easily lost after death, although some trace (scars) of the peribuccal spines may often be seen. Some authors, for example Wolfgang (1955), question the validity of the genus *Tormopsolus*. We find no trace of peribuccal spines in *Acaenodera* even when the large body spines are intact. Both the lateral and ventral enlarged spines may be lost either partially or completely. Usually some lateral spines remain as short broad stumps.

Stephanostomum robustum (MacCallum, 1917) Caballero, 1952, is probably a related species. Many details of structure as sucker ratio, position of gonads, length of cirrus sac and metraterm, extent of vitellaria, egg size, etc. agree with our material. Furthermore, favorable eggs show one end bearing a sharp point, and a uroproct appears to be present (as noted by Caballero). However, *S. robustum* is much larger; an immature specimen is at least 4 times larger than some mature Hawaiian specimens and there are small differences in the terminal genital ducts. Peribuccal spines were lacking in *S. robustum* but presumed lost. The type slide of *S. robustum* was kindly loaned by the U.S. National Museum. The 8 specimens were much flattened in killing and are somewhat macerated as noted by both MacCallum (1917) and Caballero (1952). Caballero believed some of the specimens to be *S. hispidum*, but the variations involved seem to us to be individual variations and we believe only one species is represented. There is reason to believe that this species once had enlarged spines on the forebody and probably belongs in the genus *Acaenodera*. No trace of peribuccal spines could be seen. Both MacCallum and Caballero refer to 2 kinds of cuticular spines on the forebody. These are in addition to the vesicular swellings probably due to maceration and appear to be the stumps or rudiments of lateral spines or plates. Some Hawaiian specimens show similar remnants of these spines. Final disposition of *S. robustum* must depend on collection of better material from the conger eel in the Atlantic.

SUMMARY

One new genus and 4 new species of digenetic trematodes are described from 6 species (4 families) of eels found in Hawaii, as follows: *Acaenodera placophora* n. gen., n. sp. and *Dolichoenterum microcotylum* n. sp. from *Conger cinereus* Ruppell; *Helicometra dochmosorchis* n. sp. and *Helicometrina quadrorchis* n. sp. from *Gymnothorax buroensis* Bleeker, the latter also from *G. steindachneri* Jordan and Evermann.

Opegaster ditrematis Yamaguti, 1942, is reported from *Moringua javanica* (Kaup) and *Myrichthys maculosus* (Cuvier); new distribution and host records.

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Explanation of Plates

All figures drawn with the aid of a camera lucida; value of projected scale indicated in millimeters. Abbreviations used: a, anus; ce, cecum; cs, cirrus sac; ej, ejaculatory duct; ep, excretory pore; ev, excretory vesicle; ga, genital atrium; gp, genital pore; hd, hermaphroditic duct; lc, Laurer's canal; m, mouth; mt, metraterm; ov, ovary; pp, pars prostatica; sr, seminal receptacle; sv, seminal vesicle; t, testis; u, proproct; ut, uterus; yr, yolk reservoir.

Plate I

Figure 1. *Dolichoenterum microcotylum* from *Conger cinereus*, semilateral view of holotype.

Figure 2. *D. microcotylum*, oral sucker of paratype.

Figure 3. *D. microcotylum*, dorsal view of anterior sucker.

Figure 4. *D. microcotylum*, ventral view of terminal genital ducts.

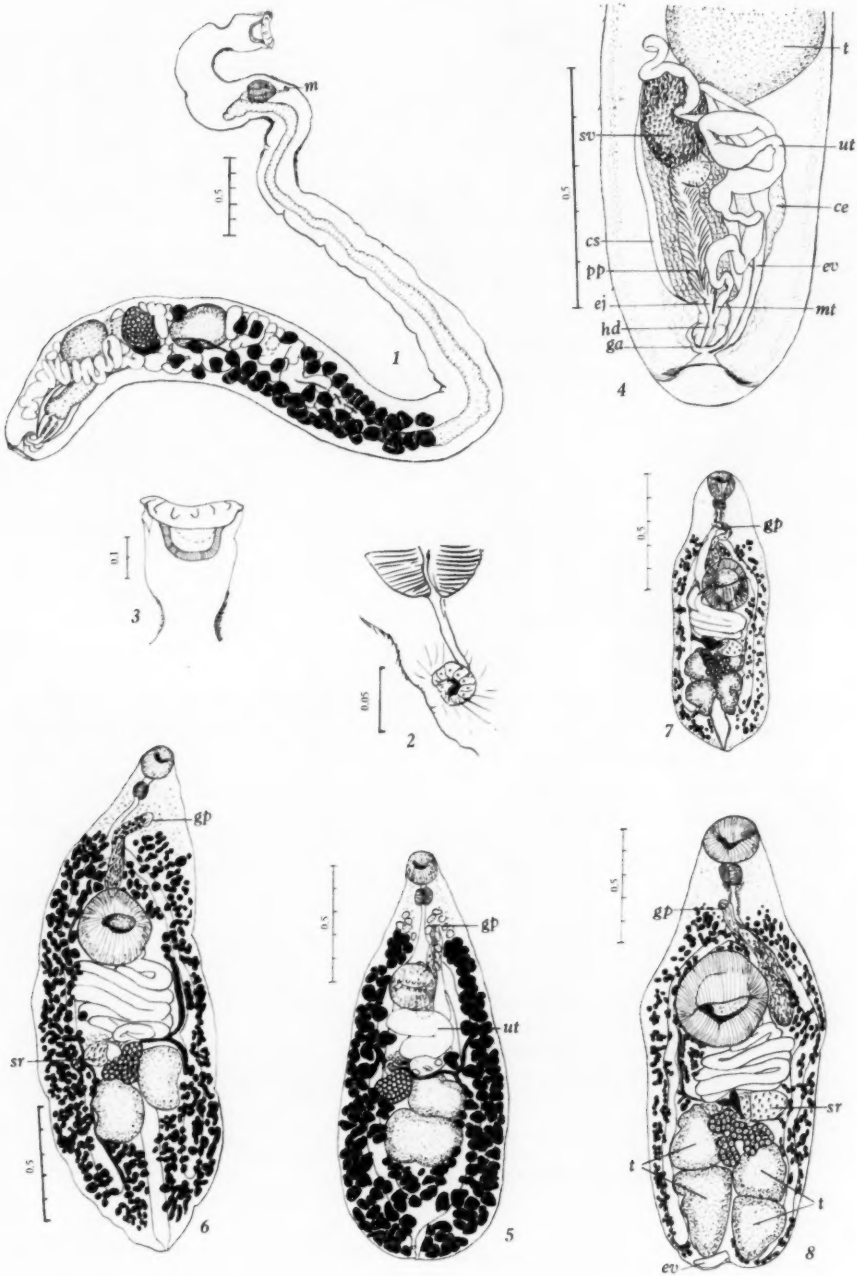
Figure 5. *Opegaster ditrematis* Yamaguti, 1942 from *Moringua javanica*, ventral view.

Figure 6. *Helicometra dochmosorchis* from *Gymnothorax buroensis*, ventral view of holotype.

Figure 7. *Helicometrina quadrorchis* from *Gymnothorax buroensis*, ventral view of holotype.

Figure 8. *H. quadrorchis* from *Gymnothorax steindachneri*, ventral view of paratype (note scale same as Fig. 7).

PLATE I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gp
sr
t
t
ev
8

PLATE II

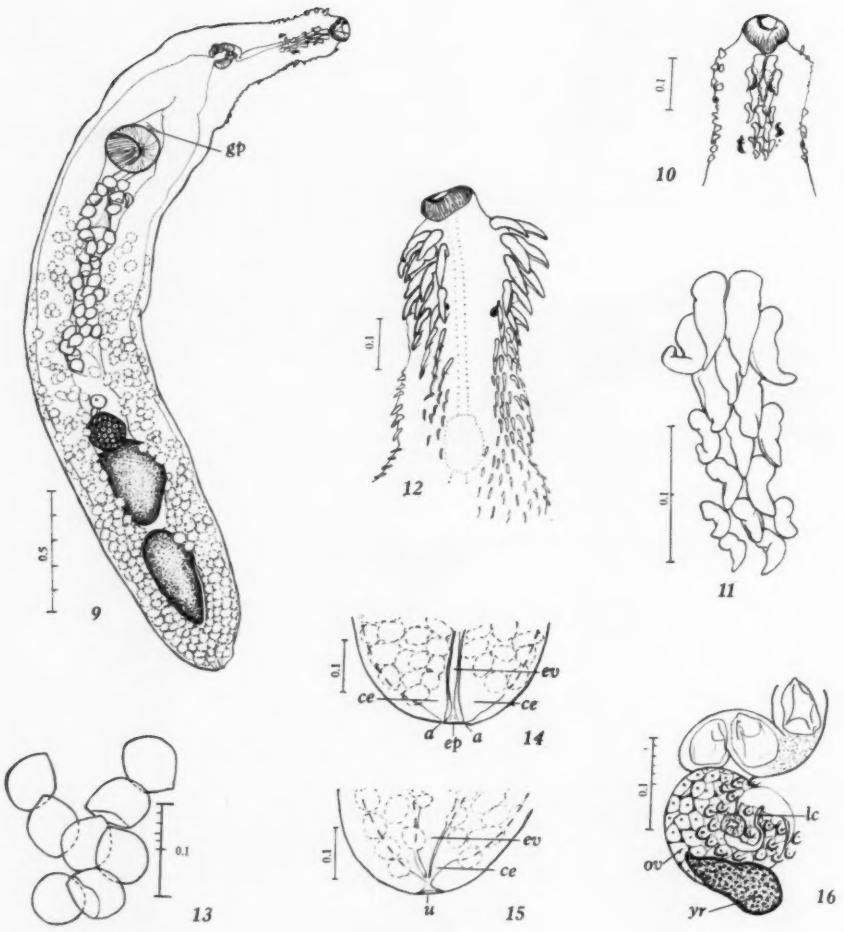

PLATE II

FIGURES 9–16. *Acaenodera placophora* from *Conger cinereus*.
FIGURE 9. Ventral view of holotype.
FIGURE 10. Ventral view of anterior end of holotype.
FIGURE 11. Ventral plates.
FIGURE 12. Dorsal view of paratype showing lateral spines.
FIGURE 13. Eggs.
FIGURE 14. Posterior end of body showing ani (holotype).
FIGURE 15. Posterior end of body showing uroproct (paratype).
FIGURE 16. Dorsal view of region of Laurer's canal.

THE INCIDENCE OF LIVER FLUKES (*FASCIOLA GIGANTICA*) AND HYDATID CYSTS (*ECHINOCOCCUS GRANULOSUS*) IN KENYA CATTLE

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During a previous survey (Froyd, 1959a and b) a former assistant, Mrs. B. E. Rogoff, noticed that there were remarkably few hydatid cysts in the livers of cattle infected with liver flukes. It was suspected that inspection had failed to reveal the full extent of hydatid infection in livers containing flukes, due, possibly, to human error or some other cause. A further investigation was therefore undertaken.

PROCEDURE

The Kenya Meat Commission Abattoir at Athi River was visited 7 times and the livers, spleens, hearts, and lungs from all animals being slaughtered were examined thoroughly for the presence of these parasites; the only exceptions made were to take not more than 50 beasts from any one farm, thus covering as large a number of farms as possible within the limit of 1,000 head, and to exclude animals when there was uncertainty that liver and lungs belonged to the same beast. Livers were inspected by cutting the thin left lobe twice and making a deep parallel cut into the right lobe with a number of small sub-cuts; both surfaces were examined carefully. Lungs were examined by palpation and incision and spleens by visual examination.

The cattle came from 45 farms in various parts of Kenya and 3 African areas.

RESULTS

Liver flukes (*Fasciola gigantica*) or "pipestem" damage due to old fluke lesions were observed in 341 livers and lesions in the lungs of 5 beasts were attributed to flukes; the latter occurred in beasts with infected livers and were not included in the calculations. The comparative figure in the previous survey was 354 (Froyd, 1959a). Hydatid cysts were currently found in 302 beasts compared with 255/1,000 last time (Froyd, 1959b). An analysis of the incidence of hydatid cysts in lungs and livers according to whether the livers contained flukes or not gave the results shown in table I.

TABLE I. *Distribution of hydatid cysts in the presence or absence of liver flukes.*

Hydatid cysts	Fluke Absent		Fluke Present		Total	Change when present VI (pct.)	Statistical significance VII
	I	II pct.	III	IV pct.	V		
a. Total incidence	215/659	32.6	87/341	25.5	302	7.1 less	$P \simeq 0.02$
b. Total in liver	159/659	24.1	45/341	13.2	204	10.9 less	$P < 0.001$
c. Total in lungs	118/659	17.9	56/341	16.4	174	1.5 less	Not signif.
d. Incidence in both sites	62/659	9.4	14/341	4.1	76*	5.3 less	$P < 0.01$
e. Incidence in livers only	97/659	14.7	31/341	9.1	128*	5.6 less	$P < 0.02$
f. Incidence in lungs only	56/659	8.5	42/341	12.3	98*	3.8 more	$P < 0.1$
TOTALS	215/659		87/341				> 0.05

Flukes only present 254
Neither present 444
* One in each category had hydatid cyst affecting the spleen also.

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The incidence of hydatids and flukes on the various farms was found to be randomly distributed; the number expected to be infected with both parasites was calculated as 29.4 and the observed number was 29.

Table II shows the incidence of hydatid cysts in various sites, their number, size,

TABLE II. *Number, size and state of hydatid cysts in 1,000 Kenya slaughter cattle.*

Site	Number of cysts			Size of cysts		State of cysts			Total
	Single	2–5	6 and over	Large	Small *	Fresh ***	Calcified or very small **	Mixed	
Liver only	78	27	22	12	115	25	98	4	127
" " pct.	61.4	21.3	17.3	9.4	90.6	19.7	77.2	3.1	
Lung only	80	11	6	49	48	68	28	1	97
" " pct.	82.5	11.3	6.2	50.5	49.5	70.1	28.9	1	
Lung and liver : liver	37	23	15	16	59	18	54	3	
" " " " pct.	49.3	30.7	20.0	21.3	78.7	24.0	72.0	4.0	
Liver and lung : lung	39	17	19	47	28	57	16	2	75
" " " " pct.	52.0	22.7	25.3	62.7	37.3	76.0	21.3	2.7	
Liver and spleen : liver			1	1			1		1
Lung and spleen : lung			1	1		1			1
Liver, lung and spleen : liver	1				1		1		1
Liver, lung and spleen : lung	1				1		1		1
Total liver	116	50	38	29	175	41	156	7	204
" " pct.	56.9	24.5	18.6	14.2	85.8	20.1	76.5	3.4	
Total lung	120	28	26	97	77	126	45	3	174
" " pct.	69.0	16.1	14.9	55.7	44.3	72.4	25.9	1.7	

* Small cysts under 5 mm. ** Possibly very young. *** Containing fluid.

and state of development.

In those animals with both organs affected, the combination of fresh and calcified cysts were as follows: 11 animals had calcified cysts in both organs; in 10, cysts were fresh in both; 43 had calcified or very small liver parasites, but fresh ones in the lungs; 7 contained a mixture, and in only 4 were the cysts fresh in the liver but calcified in the lungs.

DISCUSSION

The observed total incidence of fluke having been 341/1,000 and that of hydatid 302/1,000, the expected incidence of both together was $341 \times 302/1{,}000 = 103/1{,}000$ or 10.3 percent; incidence of double infection was 16/1,000 below expectation. Using a chi-square test on the above data, $X^2 = 5.404$ and $P = 0.02$ approximately. The reduction may have been due to interference by either organism with the establishment of the other. It is seen from table I that there was a difference of 7.1 percent or just over one-fifth, in incidence of hydatids between cattle without and cattle with flukes, i.e. flukes appeared to prevent the establishment of 22 percent of the expected hydatids. The converse is equally likely; hydatid prevented flukes. Flukes were present in 698 hydatid free cattle (36.4 percent) whilst in 302 infected ones the rate dropped to 28.8 percent, again a drop of just over one-fifth. There is no evidence at present to show which of these two processes occurs and it is possible that it depends on which parasite enters first. [Servantie (1921) found that serum from a patient suffering from fascioliasis cross-reacted in a complement fixation test with fluid from a sheep's hydatid cyst used as an antigen, thus showing some relationship between these two parasites.]

A calculation of the change of incidence in hydatid infection in the presence or absence of liver flukes is shown in table I, column VI, with the significance of the difference in column VII. There was a very significant drop in the total number

of hydatids in the liver when liver flukes were present, but no significant difference in the lungs in the presence or absence of fluke (VIIc). The above shows that the total incidence of liver hydatids in fluke-infested cattle dropped by 10.9 percent, a fall of 45 percent from the norm when they were absent. Of this, 5.6 percent occurred among cattle with liver infections only, whilst the remainder occurred among stock with simultaneous lung hydatids. Furthermore, the liver hydatids were reduced quite irrespective of whether a lung infection existed or not. Lung hydatids showed a small insignificant reduction amongst the fluke infested cattle, probably due to random variation. The incidence of lung infections only was, of course, increased, for some of the potential 2-site infections become lung-only as a result of the drop in the total incidence of liver infections.

The expected incidence of hydatids in both sites in fluke-free cattle was 28.5/659 or 4.3 percent, whilst the observed figure was 62/659 (9.3 percent). In fluke infected stock the respective figures were 7.4/341 (2.2 percent) and 14/341 (4.1 percent). It was thus shown that there were significantly more double infections in lungs and livers than single-site ones, than a calculation would lead one to expect. From the nature of infection with hydatid cysts, as far as this is known, this result may be hardly surprising.

From table II it is possible to calculate various ratios of occurrence of hydatid to compare with figures given by Pullar and Marshall (1958); the figures for the previous Kenya survey (Froyd, 1959b), are given for comparison, and agree closely with the current survey:

	This Survey	*Previous Kenya Survey*
Total liver: total infected	204: 302 = 1: 1.48	1: 1.52
Total liver: total lung	204: 174 = 1: 0.85	1: 0.79
Liver only: lung only	127: 97 = 1: 0.76	1: 0.71

As shown in table II, single cyst infections exceeded multiple infections when one organ alone was parasitised, but where both contained cysts the ratio was reduced and the proportion was then roughly 1: 1.

The proportion of small cysts in the liver (86 percent) was significantly greater than the proportion of small cysts in the lungs (44 percent); this may be due to the physical or physiological-anatomical nature of the respective organs. According to Jenkins (1949), the vascular supply of the host tissue is of paramount importance in determining the growth rate of the parasite; more rapid growth occurred in the liver of young children. Density of the surrounding structure might check the rate of growth. It may be that some such physical factors occur in cattle and pressure of the adjoining rumen limits the growth of cysts.

There was also a significantly higher proportion of calcified cysts in the liver (76 percent) than in the lungs (26 percent) irrespective of whether they were single or two-site infections.

SUMMARY AND CONCLUSIONS

A survey on 1,000 slaughter cattle examined particularly for liver flukes and hydatid cysts showed that:

1. There was an incidence of about 34 percent flukes and 30 percent hydatids.
2. An interference between the two parasites occurred only in the livers, and was not affected by the presence of lung hydatids; i.e. the one lowered the incidence of the other.

3. The ratios of infections with hydatids in livers and lungs corresponded closely with a previous survey.

4. Information on the number, size and state of hydatid cysts is given and results are briefly discussed.

ACKNOWLEDGMENTS

Thanks are due to the Kenya Meat Commission for providing facilities for this work, to Mr. P. Scoggins for his help in collecting the data, and to Mr. E. Whiteside for carrying out the statistical analysis. Permission from the Director of Veterinary Services, Kenya, to publish this paper is acknowledged.

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RESEARCH NOTE

ON THE LIFE CYCLE OF *MEGALODISCUS MICROPHAGUS* INGLES (TREMATODA: PARAMPHISTOMATIDAE).

The trematode *Megalodiscus microphagus* Ingles, 1936, described originally from *Bufo boreas* Baird and Girard from northern California, is common in amphibia of the Pacific Northwest. In a series of *Hyla regilla* Baird and Girard taken near Portland, Oregon, 35 percent were infected, whereas 56 percent of *Rana aurora* Baird and Girard and 96 percent of *Taricha granulosa* (Skilton) from a large pond near the Clackamas River, 70 miles southeast of Portland, harbored this parasite. In addition, it has been found in *T. granulosa* from Lincoln County along the Oregon coast, and in the same species of host at Cascade Lake, Orcas Island, Washington.

The small snails *Menetus cooperi* F. C. Baker and *Gyraulus* sp. were found to serve as intermediate hosts. The redia is comparable in size to that of *M. temperatus* (Stafford) as described by Krull (1932, Occ. Papers Mus. Zool., Univ. Mich., No. 237); but the body of the cercaria is larger, since after being killed in hot 10 percent formalin it averages 0.75 mm long by 0.31 mm wide.

Emergence of cercariae from the snail is sporadic although it was not found to occur at night. During a period of 3 days, five snails yielded a total of 3, 11, 21, 24, and 33 cercariae, respectively, compared to the much greater cercarial output of *M. temporatus* from the larger *Helisoma trivolvis*.

As with *M. temporatus*, encystment often occurred upon the skin of tadpoles or on the skin of adult amphibians, or on glass and other inorganic materials. However, infection was commonly brought about in tadpoles by the swallowing of cercariae, apparently without encystment. Dissection of hosts at successive intervals after swallowing of cercariae did not disclose cysts, but only tailless bodies little larger than the bodies of the cercariae. Twelve small *M. microphagus*, averaging 1.6 mm long, with eye-spots and well-developed sex organs but no eggs, were found in the intestines of large, laboratory-reared tadpoles of *Hyla regilla* 1 month after exposure to cercariae. In other experiments, the worms had lost their eye-spots after 6 weeks and had only a few eggs after 7 weeks; others had no eggs after 2 months. The largest of the specimens with eggs was 2.4 mm long, whereas those 2 months old averaged 2.1 mm long.

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STUDIES ON THREE HEMIUROID CERCARIAE FROM FRIDAY HARBOR, WASHINGTON*

HILDA LEI CHING**

Life cycles have been traced for only a few species of the trematode superfamily Hemiuroidea: *Halipegus occidualis* by Krull (1935), *H. eccentricus* by Thomas (1939), *H. amherstensis* by Rankin (1944), *Bunocotyle cingulata* by Chabaud and Biguet (1955), and *Lecithaster confusus* by Hunninen and Cable (1943). The first intermediate hosts of *Halipegus* species are fresh water gastropods. The second intermediate hosts may be dragon fly nymphs, copepods, and ostracods (Macy and Demott, 1957). Adults of *Halipegus* occur in amphibians. Chabaud and Biguet (1955) found larval infections of *Bunocotyle cingulata* in brackish water mollusks. The metacercariae of this species become progenetic in copepods, but adults have been reported from fresh-water fishes, according to Yamaguti (1958). *Lecithaster confusus,* the only marine species whose life cycle is known, utilizes gastropods and copepods as intermediate hosts; the adult occurs in marine fishes.

The development of cercariae in the gastropod host and the infection of the second intermediate host have usually been described in reports of life cycles. In addition, Dollfus (1950) described the embryology of *Cercaria calliostomae* in detail and Hussey (1941) followed the development of the excretory system in the cercaria of *Halipegus occidualis.*

To date, 49 hemiuroid (cystophorous type) cercariae have been named, 27 from fresh water mollusks and 22 from marine. To the 40 species and their hosts listed by Dollfus (1950), Chabaud and Biguet (1955) added 5 more and also described the cercaria of *Bunocotyle cingulata.* To this list may be added *Cercaria dollfusi* Arvy, 1951, from *Philine aperta* in France, *Cercaria longicerca* Ito, 1953, from *Semisulcospira* sp. in Japan and *Cercaria caribbea XXXIV* Cable, 1956, from *Pyrene mercatoria* in Puerto Rico.

While copepods may serve as second intermediate hosts of marine hemiuroids, Lebour (1917), Linton (1927), and Myers (1955, 1956) reported larval forms in chaetognaths. Lloyd (1938) found one in a ctenophore, and Uspenskaya (1955) listed *Genarches mülleri* as found in an amphipod. Progenetic hemiuroids were described from a marine shrimp by Dollfus (1927) and from a fresh-water shrimp by Yeh and Wu (1955). Chabaud and Biguet (1955) have reviewed the different methods of infection of the crustacean host.

Two marine hemiuroid cercariae briefly described as *Cercaria A* and *Cercaria B* by Miller (1925) were available for study during the summers of 1957 and 1958 at the Friday Harbor Laboratories of the University of Washington. Another such cercaria was found in 1958 and identified as *Cercaria prenanti* Arvy, 1949. Appreciation is expressed to the personnel of the laboratories for generous facilities and aid in collection, and to Dr. H. W. Manter for suggestions and criticisms. The

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study was supported by a National Science Foundation summer grant in 1957 and the Wolcott Memorial Fund of the University of Nebraska in 1958.

METHODS AND MATERIALS

Rediae and cercariae were studied in natural infections of *Cercaria A* in *Thais emarginata* (Deshayes) and *T. canaliculata* (Duclos); *Cercaria B* in *T. lamellosa* (Gmelin); and *Cercaria prenanti* in *Dentalium dalli* Pilsby and Sharp. Rediae were obtained by carefully dissecting them from the digestive gland of the mollusk. Cercariae were stained with neutral red and measured while living. Temporary glycerine mounts were made of cercariae fixed and stained with acetocarmine.

For experimental infections, naturally emerged *Cercaria A* and *C. B* were used, but *C. prenanti* was obtained for that purpose by rupturing the rediae. Mollusks infected with hemiuroids were detected by isolating them in fingerbowls for 2 days. In those snails not shedding cercariae, a small hole was made at the apex of the shell after the method of McCauley (1958). The digestive gland and gonad thus exposed were examined under the dissecting microscope; and if opaque rediae were seen through the epithelium, the snail was kept until it died or shed cercariae. Although this operation involved a high mortality rate in infected snails, it surpassed the usual isolation procedure because of the less space and time required. Snails surviving the operation longer than a month secreted fine layers of shell over the exposed areas.

Metacercariae were obtained by exposing harpactacoid copepods, *Tigriopus californicus* (Baker), to heavy concentrations of cercariae until each contained 1 to 5 metacercariae. The copepods were free of natural infections except for an occasional microphallid metacercariae.

After exposure to cercariae for 6 to 24 hours, the copepods were placed in plastic bags or fingerbowls with food in the form of green algae in solution or on small stones. The containers were placed in running water at 14 to 16 C and sea water was added to them occasionally. Periodic examination of the infected copepods was made. Metacercariae were measured and studied alive while in the copepod or dissected out in crustacean serum and as fixed and mounted specimens. All measurements are in millimeters.

In feeding experiments, metacercariae 10 to 24 days old were fed to young perch, sticklebacks, gobies and scorpaenids after samples of them had been isolated beforehand and examined for parasitic infections. Since the copepod mortality was high, only 10 to 15 metacercariae could be fed at one time to each fish. Older metacercariae were used in smaller numbers.

OBSERVATIONS

Copepods were readily infected with *Cercaria A* and *C. B*. These infections were found up to 24 days for *Cercaria A* and 16 days for *C. B*. Copepods exposed to *C. prenanti* did not become infected.

Fishes fed copepods infected with *Cercaria A* or *B* were examined carefully after 24 hours, but no metacercariae were found in the gut.

Observations on the development and morphology of each cercaria will be considered separately.

Cercaria A *Miller, 1925*

Redial infections were found in 73 or 23.5 percent of 311 snails examined. The rediae are extremely long and so entangled that it is difficult to tease out complete ones. They are sometimes almost as long as the digestive gland and give it a spotted, opaque appearance. They measure 10 to 15 by 0.5 to 0.9, and are tubular with constrictions. The pharynx is weakly muscular and only the anterior-most part of the cecum is visible. Germ balls and cercariae in all stages of development are present, but the cercarial bodies are not encysted, i.e., withdrawn into their caudal vesicles.

The cercaria (figs. 1–8) develops from the germ ball which divides into 2 parts; the larger becomes the body and the smaller the "cyst" or caudal vesicle. The primordium of the cyst develops a club-like projection which elongates and contains

about 14 cuboidal cells, the distal-most of which is largest and does not stain. The cell boundaries and nuclei of this appendage disappear and the appendage becomes a hollow tube, as the cyst acquires a double-walled cavity. This appendage is the delivery tube, or "arrow" of Sinitsin, through which the cercarial body escapes. A smaller club-shaped projection with 1 nucleus is located near the junction of the body and cyst and corresponds to the "ribbon" of Sinitsin. Its function could not be determined. The body of the cercaria is little differentiated except for the weakly muscular suckers, small pharynx, and pear shaped, thin-walled excretory vesicle. A fine excretory tubule connects a flame cell, one on each side of the body, to the excretory bladder. When the redia is ruptured, the body, arrow and ribbon of the fully developed cercaria immediately withdraw into the caudal vesicle.

How the cercariae escaped from the snail host could not be determined. Snails actively shedding them were carefully dissected and showed no ruptures of the digestive gland or kidney epithelium. Cercariae were frequently seen along the mantle and on the foot of the snail before being deposited in clusters on the sides and bottom of the dish. In fingerbowls containing fresh sea water, they lived almost 2 days and then became opaque.

The cyst is disc-shaped with the margin slightly flattened at one place. From the side, it is convex and tapering to a thin edge. The cercarial body lies in a semicircle within the cyst with the oral sucker near the coiled delivery tube. Slight pressure of the cover slip causes the delivery tube to shoot out followed by rapid passage of the cercarial body through it. Not far from the cyst, the delivery tube bears 2 diagonally-opposite projections.

Description of Cercaria A (*based on 10 live specimens*): Cercarial body elongate, blunt anteriorly, 0.132 to 0.144 by 0.018 to 0.030. Oral sucker and acetabulum faintly visible, 0.016 to 0.020 in diameter; acetabulum equatorial. Pharynx 0.006 in length followed by short intestinal stem, 0.016 to 0.020 long. Excretory bladder round to pyriform, with main stem dividing posterior to acetabulum to form a pair of delicate lateral excretory tubules each of which gives off a flame cell about midway between acetabulum and oral sucker.

Caudal vesicle round with slight bulges, when not enclosing cercarial body, 0.090 in diameter, with retracted body of larva, 0.105 to 0.115. Internal part of cyst composed of large cells which become non-nucleated and form the thin inner wall. Delivery tube 0.350 by 0.018 with enlarged tip. Club appendage 0.022 by 0.010.

While the actual projection of the delivery tube was never observed in the copepod, several times one was seen to take a cyst into its buccal cavity and, seconds later, a cercarial body was observed in the anterior end of the hemocoel or in the furcal spaces of the abdomen. It thus seems that "excystment" must project the worms directly through the wall of the gut. In heavy concentrations of cercariae and copepods, the movements and feeding activity of the copepods caused some worms to excyst in the water where they waved about actively on their posterior ends but died after 24 hours. Free cercarial bodies that may have been eaten or emerged abnormally in the copepod were found in the gut. They were not seen to penetrate it but, instead, soon died and were quickly eliminated in the copepod's feces.

Copepods were readily infected with the worms until the hemocoel became packed with them. Copepods with multiple infections lived up to 19 days; those containing one worm lived much longer and the parasite attained a larger size than did worms in heavily infected copepods.

After reaching the hemocoel of the copepod, the larvae became more and more active for 5 days and increased in length and width but showed little differentiation.

About the 5th day, a conspicuous pre-oral lip appeared. After the 19th day, the posterior end appeared contractile and irregular in shape due to eversion of part of the excretory bladder, but no ecsoma was observed at any time. The worm became more muscular and proportionately wider than in the cercarial body. At 24 days, its length was 0.35 in preserved material and as great as 0.5 in living specimens.

By the 9th day, the acetabulum was more muscular but unchanged in position and after 2 weeks, it became larger than the oral sucker and its muscular fibers continued to increase. At about 19 days, small sensory papillae were observed on the surface of both suckers; the acetabulum was more anteriorly placed and the sucker ratio was 1 : 1.5.

Outlines of the intestinal ceca anterior to the acetabulum were observed on the 5th day. The entire digestive system with the ceca reaching the posterior end could be seen on the 9th day. The pharynx had become almost 6 times as large as in the cercaria. At 24 days, the ceca contained oil globules similar to those found in the hemocoel of the copepod.

The main excretory tubules were seen to branch but the flame cell pattern could not be determined. An anterior commissure was not observed. The bladder is at first thin-walled but at 2 weeks, it and the common median stem were surrounded by cells. By the 19th day, these parts were lined with large cells. The bladder terminated in a large pore through which villus-like structures of the bladder lining were projected and withdrawn with contractions of the bladder and posterior end.

Differentiation of the reproductive system becomes evident on the 5th day when small symmetrical primordia of the testes appear posterior to the acetabulum. By the 9th day, the ovoid sinus sac midway between acetabulum and oral sucker was observed in lateral view. On the 17th day, a dark, median, granular mass, the ovario-vitelline complex, appeared posterior to the testes. By the 24th day, the vitelline primordium separates from this mass. A globular seminal vesicle slightly posterior to the acetabulum, and a faint pars prostatica joining the seminal vesicle to the sinus sac were seen in a 24-day specimen mounted in lateral aspect.

Discussion of Cercaria A: The embryology of *Cercaria A* is similar to that of other hemiuroid cercariae. The simple combination of the delivery tube and ribbon is unusual among these cercariae. The method of "excystment" is like that of *Halipegus occidualis* in the small copepods used but may be different in larger ones.

The development of the metacercaria is like that of *Halipegus eccentricus* in 2 ways. Thomas described the eversion of villi from the excretory bladder of *H. eccentricus*. This phenomenon was also observed in *H. occidualis* by Macy and Demott (1957). Thomas reported oil globules in the intestine of the metacercariae of *H. eccentricus* after the metacercariae were in the hemocoel for a few hours. The digestive system of *Cercaria A* does not develop completely until after 9 days in the copepod. Oil globules were seen in the ceca of metacercariae when they were 24 days old.

In contrast to its early development in *H. eccentricus, H. occidualis,* and *H. amherstensis,* the heavy-walled excretory bladder does not develop until the metacercariae of *Cercaria A* are in the hemocoel of the copepods for at least 2 weeks.

The metacercaria of *Cercaria A* agrees with *Lecithaster salmonis* Yamaguti, 1934 in body shape, the sucker ratio of small adults, location of the genital pore, extent of the seminal vesicle, and absence of an anterior excretory commissure. The high incidence of infection of snails by *Cercaria A* and fish hosts by *L. salmonis*

(81 from 50 fish of 8 species collected at Friday Harbor by Ching, 1960, in press) is also suggestive. If *Cercaria A* is a species of *Lecithaster,* the ovary and vitellaria must become lobed before ingestion by the final host. Such a nearly mature *Lecithaster* larva was found in a naturally infected cyclopoid copepod. Proof that *Cercaria A* is actually the larval form of *L. salmonis* awaits more experimental work and survey of naturally infected copepods.

Cercaria B *Miller, 1925*

This cercaria was reported by Miller as being immature in 3 of 4 infections of *Thais lamellosa.* Forty-one or 2.6 percent of 1568 snails examined in 2 summers by the writer were infected, but only 9 contained cercariae that were considered to be fully developed. Two snails shed cercariae during the first day only, although the rediae showed a mature, heavy infection when dissected out.

In young infections, rediae containing pigmented germ balls appeared like specks near the surface of the reddish brown digestive gland. In older infections, the rediae became long and constricted and filled the digestive gland.

Description: Cercarial body 0.083 by 0.030, oral and ventral suckers poorly developed, respectively 0.016 and 0.020 in diameter. Excretory system with round, thin bladder, common median stem dividing posterior to acetabulum to form ascending tubules which disappear between pharynx and acetabulum. Two flame cells anterior and lateral to acetabulum, their connections not observed. Caudal vesicle ovoid, with an easily lost filamentous appendage, about 0.062 in diameter when empty, 0.079 to 0.099 with cercarial body and delivery tube retracted. A delivery tube 0.266 by 0.015 developing from the base of the vesicle, with two "lateral" extensions at its base. After rupture of the redia, cercarial body and delivery tube entering the vesicle together where the body becomes C-shaped much as in preceding species. "Excystment" easily induced by cover slip pressure.

Naturally shed cercariae were placed with copepods which became readily infected. Development of metacercariae in the copepod was so similar to that of *Cercaria A* up to the 16th day that the 2 were difficult to distinguish. Only a few metacercariae were available for study and infected copepods could not be maintained beyond that day.

Cercaria B appears to be similar to the larva of *Lecithaster confusus* as described by Hunninen and Cable (1943) and its embryology corresponds to that figured by them (p. 74). Both species possess a filamentous appendage and simple delivery tube and the differences between them are minor. *Cercaria B* has very long rediae in which larvae were never found "encysted" as in *L. confusus.* The excystment process in the copepod appears to be similar but development in that host was not as rapid as described by Hunninen and Cable for their 9-day-old metacercariae which were twice the length of the present species at 2 weeks. The copepods, *T. californicus,* may not have been the optimum experimental hosts for these larvae.

None of the fishes examined for hemiuroids was infected with *L. confusus* although 8 species harbored *L. salmonis. L. confusus* is similar to *L. salmonis* except for egg size. *L. confusus* may occur in small numbers in a species of fish not yet examined. It is possible that the cercaria described by Hunninen and Cable is not the larva of *L. confusus.*

Cercaria prenanti *Arvy, 1949*

A hemiuroid cercaria was found in two of 42 *Dentalium dalli* dredged mainly from East Sound. One scaphopod was infected with 42 rediae, the other with 34.

The infection covered the digestive gland region entirely and appeared to have castrated the hosts. The rediae are compact and cylindrical, measuring 0.296 to 0.325 by 0.044 to 0.059. Each contained "unencysted" cercariae of relatively the same age although a few germ balls were present.

Description: In larvae released from the redia, the cercarial body active and contractile, measuring 0.163 to 0.192 by 0.015 to 0.030. Empty caudal vesicle 0.052 to 0.057 by 0.044, with retracted body and delivery tube 0.052 to 0.059, oral sucker muscular, ovoid; pre-oral lip present. Suckers subequal, acetabulum in posterior half of body. Pharynx spherical, 0.008 in diameter. Esophagus with slight bifurcation apparently present. Excretory bladder pear-shaped, median stem bifurcating posterior to acetabulum, ascending tubules form commissure dorsal to pharynx. No flame cells observed. Caudal vesicle more or less oval when empty, its anterior end bearing long delivery tube with enlarged pointed tip; tube entering vesicle before cercarial body is retracted and curved tightly within. "Excystment" by cover slip pressure more difficult to induce than in the other 2 species studied, but the process otherwise the same.

Arvy (1957) reported *Cercaria prenanti* from 33 of 132 *Dentalium entale* near Paramé in northwest France. The gross morphology and measurements correspond to those of my specimens. The pyriform caudal vesicle, single appendage, pre-oral lip and type of host are similar. However, the finely folded cuticle was not observed in my specimens.

C. prenanti is the only hemiuroid cercaria found in scaphopods up to the present time. The adult of this larva should be common to the north Atlantic area near France and to Puget Sound.

Natural Infections of Copepods

Besides the *Lecithaster* sp. found in a cyclopoid copepod from a plankton haul, hemiuroid metacercariae were found in *Mesamphiascus parvus* (Sars), a harpactacoid copepod on intertidal red algae. These infections were too young to be identified and intensive efforts to maintain the infected copepods were not made.

SUMMARY

1. The embryology of *Cercaria A* Miller, 1925, is described. Copepods were infected experimentally and the growth and development of the metacercaria were observed up to 24 days. Attempts to infect young fish were unsuccessful.

2. Copepods were infected with *Cercaria B* Miller, 1925, and the metacercaria was studied up to 16 days. *Cercaria B* is similar to the larva of *Lecithaster confusus.*

3. *Cercaria prenanti* Arvy, 1949, is described from a new host, *Dentalium dalli.* It did not infect copepods exposed to it.

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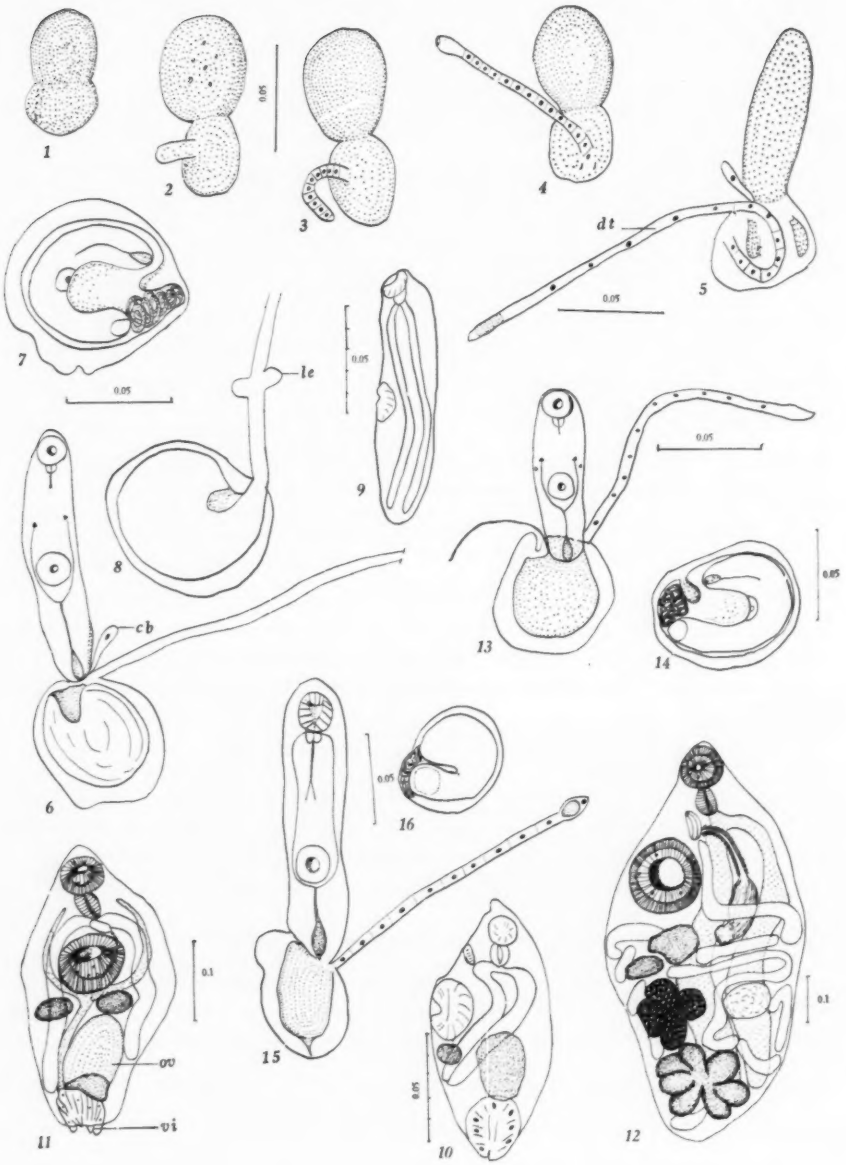
EXPLANATION OF PLATE I

All figures were drawn with the aid of a camera lucida. Scale values are indicated in millimeters.

FIGURES 1–11. *Cercaria A.*
FIGURES 1–6. Development of early larva to mature cercaria.
FIGURE 7. Cercarial cyst.
FIGURE 8. Emptied cyst showing diagonal extensions.
FIGURE 9. Metacercaria at 10 days, lateral view.
FIGURE 10. Metacercaria at 19 days, ventral view.
FIGURE 11. Metacercaria at 24 days, ventral view.
FIGURE 12. *Lecithaster salmonis,* ventral view.
FIGURE 13. *Cercaria B,* before encystment.
FIGURE 14. *Cercaria B,* cyst.
FIGURE 15. *Cercaria prenanti,* before encystment.
FIGURE 16. *C. prenanti,* cyst.

Abbreviations. cb, club appendage; dt, delivery tube; le, lateral extension; ov, ovario-vitelline complex; vi, villi of excretory bladder.

PLATE I



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RESEARCH NOTE

FURTHER OBSERVATIONS ON *THYSANOSOMA ACTINIOIDES* IN THE AMERICAN PRONGHORN.

Allen and Kyles (1953, Proc. Helm. Soc. Wash. **20:** 96–97) reviewed the literature relative to the occurrence of the fringed tapeworm, *Thysanosoma actinioides,* in the American pronghorn, *Antilocapra americana* (sometimes called the Rocky Mountain "antelope"), and recorded the parasite from this host for the first time in New Mexico. Their results showed that only two of 19 pronghorns examined harbored this tapeworm. This was in marked contrast to the incidence of fringed tapeworm in domestic sheep in New Mexico as reported by Allen and Jackson (1953, Vet. Med. **48:** 352–354) ; and it was, therefore, suggested that this parasite may not be as well adapted to pronghorns as it is to domestic sheep. Later, Gilmore and Allen (1960, Proc. Helm. Soc. Wash. **27:** 69–73) apparently confirmed this conclusion, since they found fringed tapeworm in only one of eight pronghorns that they examined, thus bringing the total number of New Mexico pronghorns examined to 27, only three of which harbored fringed tapeworms. Two of these pronghorns had one fringed tapeworm each, whereas the third had 38.

In October 1959, the present writers in cooperation with the New Mexico Agricultural Experiment Station and the Department of Game and Fish examined for internal parasites nine pronghorns that had been killed by hunters on a sheep ranch near Vaughn, New Mexico. Four sets of viscera were examined collectively, and numerous specimens of *T. actinioides* were recovered from them. Five sets of viscera were then examined individually, four of which contained rather large numbers of fringed tapeworms. These findings, contrary to those reported earlier, showed that the pronghorn could be heavily parasitized by this tapeworm. Since the host from which the fringed tapeworm was originally described was a South American deer, *Cervus dichotomus,* the present findings indicate that the original definitive host of this parasite in North America may also have been a wild ruminant.—Rex W. Allen and Kay S. Samson, *Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, University Park, New Mexico.*

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Conference Room 7

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9:00 A.M.

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SESSION A: Reports 1–14 (15, 16 by Title)
Harold W. Brown, Columbia University, Presiding

Conference Room 1

2:00 P.M.

SESSION A: Reports 17–28 (21–31 by Title)
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SESSION A: Reports 32–43
Clark P. Read, Rice Institute, Presiding
Conference Room 1

7:30 P.M.
DEMONSTRATION SESSION: Reports 44–60 (Refreshments)
Galeria Room

FRIDAY, NOVEMBER 4
9:00 A.M.
SESSION A: Reports 61–74 (75 by Title)
Donald V. Moore, University of Texas, Presiding
Conference Room 1

SESSION B: Reports 76–87 (83 by Title)
Leo Jackowski, Jr., Naval Medical Research Institute, Presiding
Conference Room 2

2:00 P.M.
SESSION A: Reports 88–101 (102–109 by Title)
David T. Clark, Michigan State University, Presiding
Conference Room 1

SESSION B: Reports 110–122 (117 by Title)
Dale A. Porter, Regional Laboratory, A.R.S., U.S.D.A., Presiding
Conference Room 2

SATURDAY, NOVEMBER 5
9:00 A.M.
SESSION A: Reports 123–139
Marietta Voge, University of California, Los Angeles, Presiding
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ABSTRACTS

1. **Comments on the Life Cycle of *Histiosoma murchiei* (Hughes and Jackson, 1958) (Acarina: Anoetidae).** J. H. OLIVER, JR. The University of Kansas.

Studies were conducted during the summers of 1958 to 1959 at the University of Michigan Biological Station on the mite *Histiosoma murchiei*. This mite parasitizes cocoons of several species of earthworms although *Allolobophora chlorotica* seems to be the chief host.

In considering the life cycle of this mite, it should be kept in mind that all stages of this parasite occur inside of the earthworm cocoon. They remain in this habitat except for a period when the hypopi leave the old cocoon and infest new ones. There are two types of developmental cycles: (1) The male cycle; and (2) the female cycle. The female goes through an egg, larval, protonymphal, hypopal, tritonymphal, and adult stage whereas the male omits the hypopal stage and goes through the other immature stages at an accelerated rate.

All mites which hatch from eggs laid by virgin females develop into males and all mites which hatch from eggs laid by mated females develop into females. It is thus assumed that sex determination depends on whether or not the eggs are fertilized and it is further assumed that the males are haploid and the females are diploid.

2. **The Development of *Porocephalus crotali* Humboldt (Pentastomida) in Experimental Intermediate Hosts.** J. H. ESSLINGER, Tulane University, School of Medicine.

Gravid female *Porocephalus crotali* were obtained from the lungs of *Agkistrodon piscivorus leucostoma* from Louisiana. Eggs shed by the parasites were administered per os to albino rats and mice, which were then sacrificed at weekly intervals over a period of 6 months. Cysts in the viscera and mesenteries containing individual parasites were dissected or were macerated in 10 percent KOH. Particular attention was fixed on the presence of cast-off cuticula within the cysts. Although entire exuviae were seldom obtained, the presence in each stage of a characteristic chitinous oral ring permitted a determination of the number of molts occurring within a cyst.

Seven developmental stages were found in the mammalian intermediate host. The first was the "primary larva", the four-legged infective stage which hatched from the egg, migrated through the wall of the small intestine, and became established on the viscera and mesenteries. This was followed by six molts, resulting in legless nymphal stages of progressively increasing size and anatomical complexity. The sixth nymphal stage, showing marked sexual differentiation and being infective to the snake, was reached in about 3 months.

3. **Sex Determination in Some Parasitic Dermanyssidae (Mesostigmata: Acarina).** J. H. CAMIN AND J. H. OLIVER, The University of Kansas.

In the few dermanyssid mite species that have been investigated, sex determination appears to be of the haplo-diploid type, with males being produced parthenogenetically from unfertilized eggs and females from fertilized eggs. Preliminary investigations have demonstrated that the *n* number of chromosomes in *Ophionyssuss natricis* is 9, the *2n* number is 18. In *Dermanyssus gallinae*, with approximately the same amount of chromatin material as in *O. natricis*, the haploid number is 3 and the diploid number is 6. Experiments are being conducted to determine whether parthenogenetic male eggs are produced intermittently, as in some Hymenoptera, or whether the production of males is merely a function of depletion of the sperm supply in the spermatheca.

4. **Microbial Isolations from the Mid-Gut of *Tenebrio molitor* L. Larvae.** G. WISTREICH AND J. MOORE, University of California, Los Angeles.

Mid-gut sections of 18 surface-sterilized, laboratory-colonized *Tenebrio molitor* L. larvae were examined with reference to microbial content. Pieces of the abdominal wall used as controls were sterile. Thirty-seven biochemical tests as well as antibiotic sensitivity tests were performed. *Streptococcus* was the most frequently encountered genus. Yeasts and fungi were not found. Food and other products in the immediate environment were also examined for comparison with the intestinal flora. (This investigation was supported in part by a Grant E–87 N.I.A.I.D., U. S. Public Health Service to Dr. G. H. Ball and a U. S. Public Health Service Training Grant (2E–70) in Microbiology.)

5. **Microbial Isolations from the Mid-Gut of the Fourth-Instar Larvae of *Anopheles albimanus* Wiedemann, *Culex quinquefasciatus* Say, and *Aedes sierrensis* Ludlow.** G. A. WISTREICH AND J. CHAO, University of California, Los Angeles.

Mid-gut sections from fourth-instar larvae of laboratory colonized *Anopheles albimanus, Culex quinquefasciatus,* and *Aedes sierrensis* were studied in relation to microbial content. *Pro-*

teus rettgeri (Hadley, et al), *Bacillus circulans* (Jordon), *Paracolobacterium intermedium* (Borman, et al), and an unidentified gram-negative rod were cultured from *A. albimanus*. *Bacillus* sp. was isolated from *C. quinquefasciatus*. *Bacillus* sp. and *Pseudomonas* sp. were isolated from *Ae. sierrensis*. The yeast *Saccharomyces* sp. was found to be present in all the three species, though less frequently in *A. albimanus*. Isolations from the water samples of larval pans showed the presence of *Aerobacter cloacae* (Jordan), *Klebsiella* sp., *Pseudomonas* sp., unidentified gram-positive and gram-negative rods, and the yeast *Saccharomyces* sp. (This investigation was supported by a grant E-87 to Dr. G. H. Ball, from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health.)

6. **Electron Microscope Observations on Microorganisms of Adult *Culex fatigans*.** D. W. MICKS, S. R. JULIAN, JR. AND M. J. FERGUSON, University of Texas Medical Branch, Galveston.

Electron microscope examination of sections (0.025 microns) of the mid-gut of adult female *C. fatigans* revealed the presence of small intracellular and extracellular rods approximating the size of mitochondria and showing ultra-structure consisting of membranes, nucleus and inclusions. In several sections, these microorganisms were grouped together intracellularly in a mycetome-like arrangement. These stain as gram-negative rods. The detailed structure of these and bacteria present in ultrathin methyl methacrylate sections will be discussed.

7. **Age Estimation of Mosquitoes.** B. ROSAY AND A. R. BARR, California State Department of Public Health, Fresno.

Techniques for estimating the age of mosquitoes, originally discovered by workers in the Soviet Union and in Africa, have been confirmed and refined. These are based chiefly on changes which take place in the ovaries of female mosquitoes as they age. Supplementary information is gained from examination of the gut, spermathecae, and remnants of muscles which persist for a short while in adult mosquitoes. These findings provide a new approach to the study of mosquito populations under natural conditions. The application of these techniques to the solving of problem situations is demonstrated.

8. **Water Balance in Mites.** G. W. WHARTON, University of Maryland.

Mites are the smallest of terrestrial arthropods and, therefore, as a group have the most difficult problem of retaining and obtaining sufficient water for their metabolism. In those species that have been investigated, their behavior is dominated by problems associated with retention and imbibition of water. The most commonly used methods of concentrating and collecting acarines are those, such as the Berlese funnel, that force the mites from material by drying it out with mild heating. In considering water balance in these small forms, techniques for the determination of water loss and water uptake have been developed. New techniques must now be devised for testing hypotheses concerning the mechanisms that enable mites to avoid desiccation in the arid atmospheres to which they are at times exposed. (This investigation was supported by a research grant (E-2622) from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health.)

9. **The Control of the Spinose Ear Tick, *Otobius megnini* (Duges, 1883), Infesting Horses, Mules and Cattle with 2 Percent Dibrom-SG 67.** I. B. TARSHIS, The University of California, Los Angeles.

The sorptive dust SG 67, impregnated with 2 percent Dibrom 8 Emulsive, was found to be highly effective for the control of the spinose ear tick, *Otobius megnini* (Duges, 1883), found infesting thorobred and Arabian horses, mules, and purebred Pollard herefords. Though all the infested animals had been previously treated on numerous occasions with a number of standardly used preparations, the ticks infesting the animals' ears had not been eliminated nor appreciably reduced by any of these preparations. Each ear of each infested animal was dusted with one-fourth to one-half ounce of 2 percent Dibrom-SG 67 applied with a long-spout hand duster. The spout was placed deep into the ear and the pinna held around the duster as the dust was released. The quick knockdown action of the Dibrom caused a number of ticks to become dislodged from the ears shortly after the dustings. These ticks were well covered with the dust and completely immobilized. The dust did not have any adverse effects on the host animals. On week after dusting, the ears of the animals were examined and all were found free of ticks. Additionally, the ears were found to have a fair quantity of the dust still within them. All treated animals remained free of ticks for 28 days without being redusted and then became reinfested, since the pastures and paddocks were untreated. However, the degree of reinfestation was slight compared to the original infestations. Subsequent dustings at 10-day intervals have kept the animals free of ticks.

10. **Factors in the In Vitro Survival of Gregarine Parasites of** *Tenebrio molitor* **L.** J. MOORE, University of California, Los Angeles.

Gregarine trophozoites from the mid-gut of *Tenebrio molitor* L. larvae remained active up to 3 days in a complex basal medium. The presence of chick serum in the medium was found not to be essential and possibly inhibitory. Substitution of a saline extract of *Tenebrio* larvae prolonged activity two-fold. Survival time was further increased by lowering the pH from 6.8 to 6.1, which closely approximates the pH of the host mid-gut. The effects of increasing the osmotic pressure of the medium to duplicate that of the host is being investigated. (This work was supported by a U. S. Public Health Service Training Grant (2E-70) in Microbiology.)

11. **The Effects of Temperature on the Oocysts of** *Plasmodium relictum* **in** *Culex tarsalis* **stored at 4 C.** J. CHAO AND G. H. BALL, University of California, Los Angeles.

Culex tarsalis were stored at 4 C with food 3 days after biting a canary infected with *Plasmodium relictum.* Some of these mosquitoes were examined for oocyst development at weekly intervals immediately after being removed from storage. Others were examined after an additional period of 8 to 10 days at room temperature, a period sufficient for normal oocyst maturation. The results showed that oocyst development was arrested at 4 C, but was resumed at room temperature as evidenced by the production of motile sporozoites, if the period of refrigeration did not exceed 2 weeks.

Only one mosquito survived 3 weeks of refrigeration; after being kept at room temperature for 10 days, it was examined for sporozoites. It was found that the oocysts had not developed beyond the stage found in the mosquitoes prior to storage at 4 C. This indicates that storage at 4 C for 2 weeks does not prevent the oocysts from developing to maturity, when the mosquitoes are returned to room temperature, but that exposure to 4 C for 3 weeks does have a damaging effect on the maturation of the oocysts. (This investigation was supported in part by a grant E-87 for the National Institute of Allergy and Infectious Diseases of the National Institutes of Health.)

12. **Further Studies on** *Trypanosoma cruzi* **in Louisiana.** R. G. YAEGER AND A. D'ALESSANDRO BACIGALUPO, Tulane University, School of Medicine.

Triatoma sanguisuga were collected from an area in which raccoons and opossums, infected with *Trypanosoma cruzi,* had been taken previously. Metacyclic trypanosomes and crithidia stages were found in feces from adults and nymphs. When feces from the naturally infected bugs were inoculated into mice, both slender and broad forms of *T. cruzi* were later seen in the blood. Tissues of these mice possessed typical intracellular leishmanial stages of *T. cruzi.* Several of the strains isolated from *T. sanguisuga* have been maintained in mice by serial passage, using blood removed by cardiac puncture. Cultures of blood from infected mice, using beef extract-blood agar medium, showed the typical crithidial and metacyclic trypanosomal stages usually seen in cultures of *T. cruzi.*

The finding of naturally infected triatomid bugs indicates how *T. cruzi* infection can be transmitted, in addition to congenital transmission, in the area studied. It is believed that this is the first report of naturally infected triatomid bugs east of Texas.

13. **The Protozoicidal Action of Some 5-Nitro-Furylacrylates.** F. P. FILICE, University of San Francisco.

A number of 5-nitro-furylacrylates were synthesized and their protozooicidal effects were measured by using *Trichomonas vaginalis* as the assay organism. As a first hypothesis it is possible to correlate a particular molecular configuration with the ability to inhibit this protozoon.

14. **A Preliminary Testing of an Analogue Theory of the Protozooicidal Action of 5-Nitro-Furylacrylates.** F. P. FILICE AND R. J. SEIWALD, University of San Francisco.

An assay into the examination of an analogue theory of the 5-nitro-furylacrylates was begun by testing the reversing effect of similarly constructed cellular chemicals and their intermediates. A second test of this theory was made by constructing geometrical isomeres of the active compounds to investigate correlative changes in protozooicidal activities.

15. **Comparative Antitrichomonal Activity of Furazolidone (3-(5-Nitrofurfurylideneamino)-2-Oxazolidinone) and Aminothiazol (2-Acetamido-5-Nitrothiazol) In Vitro against** *Trichomonas vaginalis* **and In Vivo Against Experimental Trichomonal Infections in Mice and Hamsters.** M. P. NATT, R. R. GUSTAFSON, L. SCHULTES AND P. MOYNIHAN, The Norwich Pharmacal Company, Norwich, N. Y.

Furazolidone, one of the trichomonacidal agents of the topical preparation Tricofuron (Eaton), and animothiazol (Tritheon, Ortho), a peroral preparation reported to have activity

against human trichomoniasis, showed relatively comparable in vitro activity against *T. vaginalis* as well as in vivo effectiveness against subcutaneously induced *T. vaginalis* abscess and fulminating *T. foetus* intraperitoneal infection in mice (Schnitzer et al, 1950, J. Parasit. **36:** 343) and *T. foetus* vaginal infection in hamsters (Kradolfer 1954, Exp. Parasit. **3:** 1).

Utilizing a two-fold serial dilution test, furazolidone and aminothiazol were trichomonacidal at minimal concentrations of 11 and 5 mcg per ml, respectively. In vivo, both compounds eradicated experimentally induced *T. vaginalis* and *T. foetus* infections in mice when administered perorally 18 hours after inoculation, at doses of 30 mg per kg twice daily for 2 days. Initiation of treatment prior to, at the time of, or after onset of infection had a direct effect on the dose required for cures. Furazolidone and aminothiazol were also capable of eradicating *T. foetus* vaginal infections in hamsters when administered perorally in single or multiple doses commencing 8 days after establishment of infection. The use of adjuvants, e.g., mucin, to enhance the virulence of an apparently avirulent strain of *T. foetus* in mice is also discussed.

16. *Tritrichomonas batrachorum* **from the Toads** *Bufo luetkeni* **and** *B. marinus.* A. Ruiz, University of Costa Rica.

Studies on trichomonads of *Bufo luetkeni* and *B. marinus* reveal the identity of those organisms with *Tritrichomonas batrachorum.* Cultures of the flagellate were made from these hosts. Observations were made on living organisms from cultures and on fixed specimens from fecal material and cultures.

The trichomonads of these two species of *Bufo* resemble each other. They have a broadly ovoidal body and measuring 10.6 (5.3 to 14.6) microns by 7.5 (4.0 to 14.0) microns in *B. luetkeni* and 11.3 (8.0 to 16.0) microns by 6.3 (4.0 to 10.6) microns in *B. marinus.* The flagellates present three anterior flagella of unequal length and a fine costa. The undulating membrane is well developed and generally shorter than the body. The axostyle is slender and projects for some distance beyond the body. The parabasal apparatus, when visible, is shaped like an inverted "V" or "Y". Undoubtedly these trichomonads fit the description of *Tritrichomonas batrachorum* by Honigberg (1953, J. Parasit. **39:** 191).

Bufo luetkeni, an extremely rare toad found so far only in Costa Rica, is reported as a new host for this flagellate.

In the various specimens of *B. marinus* we examined, only trichomonads corresponding to *T. batrachorum* have been found. We believe that *Trichomonas vitali* described by Pinto and Da Fonseca (1926, Bol. Biol. São Paulo **2:** 34) in *B. marinus* should be considered as synonymous with *T. batrachorum,* as suspected by Honigberg.

17. **Bephenium Hydroxynaphthoate: A New and Promising Gastro-intestinal Anthelmintic for Cattle.** A. M. Eisa and R. Rubin, Colorado State University.

Bephenium hydroxynaphthoate, chemically known as benzyl-dimethyl-2-phenoxyethylammonium (3-hydroxy-2-naphthoate), was administered orally to four of eight parasitized Hereford yearling calves. The dosage rate was 250 mg per kg of body weight.

A comparison of the treated and the untreated calves showed a marked reduction in the number of eggs of the gastro-intestinal worms per gram of feces within the first 24 hours following treatment in each calf. The reduction in trichostrongylid egg counts averaged 90 percent. For *Nematodirus* eggs, the reduction was 100 percent in all calves.

During the 4 days following treatment, no detrimental effect was noticed in any of the four treated calves. The appetite and temperature were normal and the animals showed no sign of depression.

No lesions that could be attributed to the drug were observed either grossly or microscopically in the gastro-intestinal tract of the treated calves.

Necropsies of both the treated and untreated calves were performed in order to determine the effect of the drug on the worm population. By comparing the total worms recovered from the treated calves with that of the untreated ones the following was observed: the reduction averaged 85 percent for *Ostertagia ostertagi,* 87 percent for *Trichostrongylus axei,* 100 percent for *Nematodirus helvetianus,* 99 percent for *Cooperia oncophora,* 100 percent for *Oesophagostomum radiatum,* and 99 percent for *Chabertia ovina.*

Statistical analysis of the results showed that the effect of treatment was highly significant at the 0.001 level.

18. **The Effects of Serotonin in Experimentally-Induced Ascariasis.** L. C. Costello, The University of Maryland.

These studies were conducted as a preliminary investigation of the possible role of amines in the systemic manifestations of ascariasis. Serotonin has a very pronounced effect on animals

infected with *Ascaris lumbricoides* var. *suum*. Rabbits infected with 10,000 to 15,000 embryonated ova were injected intravenously with 1 mg serotonin per kg body weight after 10 days. All of the animals reacted immediately. Three of six died instantaneously while the remaining three recovered after showing symptoms of mild anaphylaxis. The same level of serotonin elicited no response in uninfected animals. Infected controls were injected with creatinine which resulted in no reaction. This control was included since serotonin is in the form of serotonin creatinine sulfate. A group of infected animals was injected with 2 mg reserpine per kg. Three animals died and the remaining four exhibited reactions. The same level of reserpine elicited only the normal sedative activity in uninfected animals.

The results clearly demonstrate that infected animals are highly sensitive to a level of serotonin not effective in normal rabbits. Similar observations were made with reserpine. However, the reaction in the case of reserpine was less pronounced and quite prolonged. This was probably due to the action of reserpine in liberating bound serotonin thereby increasing the level of free serotonin.

19. **Activity of Dithiazanine Iodide Administered in Feed against Larval Infections of *Ascaris suum* in Young Pigs.** G. Brody and E. C. Wuest, Moorman Mfg. Co., Quincy, Illinois.

A laboratory screening procedure using artificially infected young weanling pigs was employed to evaluate the prophylactic properties of various chemotherapeutic agents against migrating larval infections of *Ascaris suum*. Criteria employed to ascertain the extent of ascarid larval infections included: (1) Feed consumption observations; (2) weight gains following infection; (3) presence and duration of "thumping" (produced by larval migration through the lungs); (4) degree of liver scarring observed at autopsy; (5) number of larvae recovered from small intestine at autopsy after 21 days.

Dithiazanine iodide (3,3'diethylthiadicarbocyanine iodide) at 0.04 percent in feed exhibited definite activity against migrating larvae of *Ascaris suum* as indicated by prevention of "thumping," prevention of depression of rate of feed intake following infection and by the absence of larvae in the small intestine. Liver pathology resulting from the migration of *A. suum* larvae was not prevented by Dithiazanine.

20. **Studies on the Efficacy of Dithiazanine Iodide against Migrating *Ascaris suum* Larvae in Pigs.** R. F. Shumard and J. C. Hendrix, Eli Lilly Agricultural Research Center, Greenfield, Indiana.

The first observation of the effectiveness of dithiazanine iodide against migrating *Ascaris suum* larvae was made by Dr. G. Brody, Moorman Manufacturing Company. Studies at Eli Lilly and Company indicate that dithiazanine iodide at 0.04 percent in the feed for periods of 7 to 21 days following experimental infection, inhibits larval migration, usually in the liver. Gross and histopathological observations and larval identifications made with the aid of artificial digestants at various times during the period of treatment, revealed little variation in effectiveness as long as dietary intake of the compound was adequate. Larval development was usually inhibited in the second stage, although third-stage larvae were observed.

21. **Ecology of the Fish *Gillichthys mirabilis* and Its Parasites.** R. King and E. R. Noble, University of California, Santa Barbara.

During the past 3 years, 1100 mudsuckers (*Gillichthys mirabilis*) have been collected from a California estuary, and records made of all of their parasites. Changes in salinity, temperature and plankton have been noted on a weekly or bi-weekly basis. Snails, copepods and other possible intermediate hosts have been examined. The longer such studies are continued, and the greater the numbers of hosts scrutinized each month of the year, the less confident the authors become in drawing simple conclusions. Small differences in the environmental factors of two populations of hosts, situated close together or far apart, appear to be responsible for significant differences in the parasite-mix of the two populations.

22. **The Histogenesis of Cuticle in the Cestode Genus *Taenia*.** E. L. Schiller, The Johns Hopkins University.

Several theories have been advanced concerning the homology and origin of the cuticle in trematodes and cestodes. Histological studies of *Taenia* cysticerci occurring in the livers of *Tamiasciurus hudsonicus* collected near Tununuk, N.W.T., Canada, have provided some additional information relative to the sequence of morphological events leading to cuticle formation in cyclophllidean cestodes. In this species, cuticle formation is preceded by differentiation of two types of cells directly beneath the embryonic membrane. The cells of one type are nucleated, elongated, tapered at each end and are arranged perpendicular to the body surface. Long,

branched fibrillar processes spread from the tapered end nearest the surface membrane and extend externally beyond it. Interspersed between these cells are gland-like cells which occur at relatively regular intervals. Cuticle appears to be formed by a folding of the embryonic membrane with deposition, between the interfibrillar processes within the folds, of a cement substance, presumably secreted by the gland-like cells. The results of this study suggest that the subcuticular nucleated cells represent embryonic ectoderm, a view originally proposed by Blochmann (1897, Zool. Anz. **20:** 460–463). These results tend to support the germ layer hypothesis for the process of cuticle formation in cestodes. (This work has been supported by a U. S. Public Health Service Grant No. E-1915 (C2).)

23. **Ultramicroscopic Evidences of Absorptive Function in Cestodes.** A. H. ROTHMAN, The Johns Hopkins University, School of Hygiene and Public Health.

The cuticle of several species of cestodes from three different orders has been examined with the electron microscope. Common to all the forms is a microthrix border projecting from the cuticle proper. The microtriches vary in size and shape from one species to another, but they all have a medullated portion which is continuous with the cuticular matrix. All the tapeworms studied have "porecanals" and mitochondria in the cuticle. There is also a subcuticle permeated with subcuticular canals in all the tapeworms investigated, except one. Photographs of many of the forms give static indications for the dynamic process of pinocytosis occurring at the surface of the cuticle, in the region between the microtriches. These photographs of the worm, interpreted as evidence for pinocytosis, closely resemble the photographs of pinocytosis as it occurs in the mammalian intestine. Cuticular pinocytosis, barring other mechanisms, may be of a selective nature, if the peculiarity of the tapeworm's substrates is considered. It is perhaps not surprising that the cuticle of the tapeworm which necessarily functions as its absorptive surface, should also have some morphological resemblance to absorptive surfaces of other animals and should also perform such dynamic activities of absorption as pinocytosis. The differences between the surface of tapeworms and other absorptive surfaces of animals are probably representative of a compounding of the absorptive functions and the functions of an external body covering. (This investigation was supported by a fellowship-E 6393, from the National Institute of Allergy and Infectious Diseases of the United States Public Health Service.)

24. **Observations on the Structure of Cysticercoids.** M. VOGE, University of California, Los Angeles.

Comparison of the structure of cysticercoids of *Hymenolepis diminuta, H. nana, H. citelli, Raillietina cesticillus,* and *Choanotaenia infundibulum* shows that in all of these there is extensive development of fibrous tissue which, in its staining reactions, resembles vertebrate connective tissue. While the cysticercoid of each species studied can be easily differentiated from those of the other species on the basis of histologic structure, their fundamental organization is similar. It is suggested that the basic histologic pattern of the cysticercoid may be similar in all cestodes which have a cysticercoid stage in their life cycle.

25. **Studies on Ante-Mortem Diagnostic Procedures in Bovine Cysticercosis Infections.** L. W. DEWHIRST, R. J. TRAUTMAN, W. J. PISTOR AND R. E. REED. University of Arizona, Tucson.

Preliminary studies have shown that ante-mortem diagnosis of *Cysticercus bovis* infections or beef measles is a possibility.

Weekly hematological and immunological studies were conducted on artificially infected cattle. Hematological studies revealed that the grams of hemoglobin per 100 cc of blood dropped from an average of 9.8 near the start of the experiment to an average of 6.5 (12 weeks post infection) and then rose to an average of 8.5 g by the time the animals were slaughtered (24 weeks post infection). This cycle was also reflected in the packed cell volume. Other hematological studies revealed little, if any, change.

Precipitin tests were conducted on the sera of all animals. Positive reactions were detected on the sixth week after infection and persisted with some variability until the animals were slaughtered. Some reactions were positive with an antigen dilution of 1:16,000.

Skin tests were conducted during the 5th and 18th week after infection. Reactions were read at 30 minutes, 1 hour, and 24 hours after intradermal injection of the antigen. Maximum reactions were evident at 30 minutes. Wheals ranged from 1 to 4 cm in size in the first test of 6 to 18 cm in the second test.

Skin tests were also conducted on 499 head of cattle suspected of being infected. Of these, 69 were considered positive and 430 as negative. When slaughtered, none of the 430 classed as

negative were found to harbor tapeworm cysts. In the "positive" group, only four contained viable cysticerci but most of the others harbored old calcified cysts.

26. **Studies on the Axenic Culture of the Rodent Tape-Worms,** *Hymenolepis diminuta* **and** *H. nana.* A. E. R. TAYLOR, G. H. BALL AND M. VOGE. University of Southern California, Los Angeles.

Attempts have been made to grow the beetle stages of *Hymenolepis diminuta* and *H. nana* in vitro and to induce excysted cysticercoid larvae to grow into adult tape-worms.

When the beetle stages of *H. diminuta* were kept in roller culture at 30 C in a medium containing an extract of *Tenebrio* larvae, serum and medium 199 or a salt solution, both the stage I and stage IV embryos showed signs of development. The early stages formed elongate stage II larvae and those of stage IV developed into stage V larvae.

Cysticercoid larvae of *H. diminuta* have been kept alive and infective in vitro for periods up to 7 days at 25 C or for 6 days at 30 C. The culture medium was a 1 percent glucose solution in dilute Ringer (pH 7.0). Cysticercoids of *H. nana* remained alive and infective for 5 days at 25 C and for 3 days at 30 C in the same medium. The use of more complicated media did not prolong the survival of either species.

When cysticercoid larvae of *H. diminuta* and *H. nana* were induced to excyst by digestion (using acid pepsin, followed by trypsin and sodium taurocholate solution), they remained alive in vitro at 38.5 C. The most successful medium was a complicated one used for tissue culture. The juveniles of *H. diminuta* survived for 4 days and retained their infectivity to rats. Those of *H. nana* remained alive for 9 days but were only infective to mice for the first 3 days. (This work was supported by a U. S. Public Health Service post doctoral training grant in microbiology (protozoology & parasitology) 2E-70.)

27. **Growth Dynamics of** *Hymenolepis diminuta* **in the Albino Rat as Affected by Population Density.** L. S. ROBERTS, The Johns Hopkins University, School of Hygiene and Public Health.

Growth of *H. diminuta* has been followed by measurements of length and wet weight from infection of the definitive host to 17 days post-infection. Observations on length were made at 0.25, 0.75, 1.0 days, and daily thereafter. Weight measurements were begun at 4 days. Infection doses ranging from 1 to 100 cysticercoids were used. The growth of this tapeworm apparently follows a skew logistic type growth curve with the following characteristics: There is a lag phase of 1 day or less followed by a phase of rapid exponential growth lasting 5 to 6 days. Subsequently, a long retardation phase ends in the maximal size, attained at 15 to 17 days. These characteristics are shown by the growth curves of all worms regardless of the population density. Further, there is no measurable difference in the rate of growth until the retardation phase is reached. After this point the rate of increase is drastically affected by the population density, the worms in larger infections growing more slowly to reach a smaller maximum size. The growth characteristics of worms from each population size can be expressed mathematically, and the length or weight of a group of worms from a given population at a given age can be predicted. These measurements are being correlated with morphological development.

This work is in disagreement with the conclusions of Chandler (1939, Am. J. Hyg. **29D**: 105), who felt that *Hymenolepis diminuta* grew slowly until 5 to 7 days postinfection, then rapidly thereafter. (This investigation was supported in part by a research grant E-1508, from the National Institutes of Health, United States Public Health Service, and it was carried out during the tenure of a Predoctoral Fellowship from the Institute of Allergy and Infectious Diseases, United States Public Health Service.)

28. **Comparative Antioxyurid Activities of NF-427 (1-(P-Chlorophenly)-3-(5-Nitro-2-Furyl)-2-Propen-1-One), NF-23 (Ethyl 3-(5-Nitro-2-Furyl) Acrylate) and Piperazine Citrate Against the Mouse Pinworm,** *Syphacia obvelata.* .M. P. NATT, L. SCHULTES AND R. R. GUSTAFSON, The Norwich Pharmacal Company, Norwich, N. Y.

NF-23 is reported to have an order of activity against *Syphacia obvelata* comparable to that of piperazine citrate (Natt et al, 1957, Am. J. Trop. M. Hyg. **6**: 389). In a limited clinical trial against *Enterobius vermicularis,* this nitrofuran also showed favorable activity resulting in cure rates of 78 percent, 89 percent and 94 percent at a dose of 10 mg per lb b.i.d. for 7, 10, and 14 days, respectively. However, the nausea and vomiting encountered in this study, when compared to the lack of side effects of piperazine citrate, has limited its utility as an oxyuricide. Recently another nitrofuran was found which has more activity against the mouse pinworm than NF-23 or piperazine citrate. When the antioxyurid activities of the three compounds were compared following peroral administration to mice at graded dosages for 1, 2, 4, and 8 days,

regardless of the duration of treatment, NF-427 produced higher rates of cure at lower dose levels than NF-23 or piperazine citrate. In those instances where NF-427 or piperazine citrate did not eradicate the cecal pinworms completely, the reduced pinworm population in the mice consisted essentially of immature worms. In contrast, the worm population observed in the NF-23 treated mice included both mature and immature worms. In a study comparing the antioxyurid activities of the three compounds against the developmental stage of *S. obvelata*, unlike NF-23, both NF-427 and piperazine citrate showed greater effectiveness against the mature worms as opposed to the very immature, developmental stages. This difference in activity against mature and immature worms was corrected by use of higher drug levels.

29. **Conjugation of an Anti-*Hymenolepis* Rabbit Globulin with Lissamine Rhodamine B 200.** R. M. COLEMAN AND G. B. DUTT, Russell Sage College, Troy, N. Y.

Antigens were prepared from specimens of *Hymenolepis nana* var. *fraterna*. Following a protein determination the saline antigenic extract was mixed with an equal volume of aluminum hydroxide gel. It was inoculated into rabbits either subcutaneously or intramuscularly and titered 10 days following the last inoculation.

Serum from exsanguinated rabbits was subjected to ethodin (6, 9-diamino-2-ethoxyacridine) fractionation. The protein content of immune globulins, to be coupled, was then determined. In preparing the fluorescent dye (essentially after Chadwick, McEntegart, and Nairn, 1958, Lancet 1 (7017): 412), 500 mg of rhodamine was ground together in a mortar with 1 g of phosphorus pentachloride for 5 minutes. Fifteen ml of dry acetone was then added, allowed to stand for 15 minutes, filtered, and brought back to volume with acetone and the optical density determined (610 millimicrons). The acetone solution of the sulphonyl chloride of RB 200 was used the same day. Conjugation and subsequent steps were carried out at 0 to 2 C. An equal volume of carbonate-bicarbonate buffer (0.5 M, pH 9) was added to the globulin solution. Although Chadwick et al (loc. cit.) used 0.1 ml of the dye solution per mg of protein lesser amounts (0.05 ml to 0.01 ml), as suggested by Barnhart (1959, personal communication) were used to prevent protein denaturation. Dye-protein ratios were determined and products dialized against a phosphate buffer of pH 7.2 until no dye was present in the dialysate, usually requiring about 9 days.

30. **Observations on the Life-Cycle of *Avitellina centripunctata* (Rivolta, 1874), an Anoplocephaline Cestode from Sheep and Goats.** A. M. NADAKAL, University of Southern California, Los Angeles.

Although the life-cycles of several species of anoplocephaline cestodes, which involve oribatid mites as intermediate hosts, are known, those of the *Avitellina* spp. appear to be uninvestigated. An attempt has been made, therefore, to study the life-cycle of *A. centripunctata*. Tape-worm specimens were recovered from the small intestines of sheep and goats obtained from slaughter houses, and oribatid mites of the genera *Protoschelobates* and *Trichoribates* were collected from the lawn in the vicinity of Aligarh Muslim University, India. The feeding of the mites with embryonated eggs was carried out under the laboratory conditions at 50 to 69 F and 50 to 55 percent relative humidity. Although thousands of mites were exposed to the eggs and examined over a period of the 3 years 1950 to 1953 only five onchospheres were recovered from the body cavity of the mite *Protoschelobates*, and none from *Trichoribates*. Apart from slight change in size, these onchospheres seemed not to have reached the cysticercoid stage. The difficulties encountered in isolating sufficient number of embryonated eggs from the thick fibrous capsules embedded in the paruterine organs coupled with the poor egg-productivity of the worms have been partly responsible for the discouraging results of the present studies. However, the recovery of at least a few larvae from the body cavity suggests that the mite *Protoschelobates* may serve as a vector for *A. centripunctata*.

31. **Studies on the Excretory and Nervous Systems of the Sheep Tape Worm *Avitellina centripunctata* (Rivolta, 1874).** A. M. NADAKAL, University of Southern California.

The present studies, with the help of serial sections and whole-mounts, showed that the pattern of coiling of excretory canals in the scolex is quite characteristic in *Avitellina centripunctata*. The ventral and dorsal excretory canals of the right side are confluent in the scolex as are those of the left side. The excretory system in this species differs from that of *A. goughi* (Gough, 1911, Quar. J. Micr. Sci. **56**: 317–383) in the absence of a communicating branch between the right and left internal loops in the scolex and in the presence of "nephrocytes" in association with both the ventral and dorsal excretory canals. The author has not been able to verify the observation made by Woodland (1927, Ann. Trop. Med. Parasit. **21**: 385–414) concerning the opening of paruterine organs into the ventral excretory canals. In immature

worms, the ventral excretory canals of the two sides unite at the posterior end of the strobila and open to the outside by a common excretory pore. An attempt has also been made to study the distribution and number of neurons in the "cephalic ganglionic mass." The two lateral ganglia each contain approximately 306 neurons; the dorsal and ventral, 85 each; the transverse and dorso-ventral commissures, 65 and 12, respectively. The number of neurons in the various ganglia and commissures in different specimens is subject to slight variations, but does not depart considerably from these approximate numbers.

32. **Studies on the Genus *Helicometrina* Linton, 1910 (Trematoda: Digenea: Allocreadiidae).** J. J. DEELMAN, University of California, Los Angeles.

Members of the genus *Helicometrina* are common and widely distributed trematodes of marine fish. The genus *Helicometrina* Linton, 1910, was erected to accommodate a trematode which could not be relegated to any known genus. The type species is *H. nimia* Linton, 1910. In 1937 a second species *Helicometrina elongata* Noble and Park was added to the genus. In a study of several hundred specimens recovered from a single species of host from one location, numerous intergrades between the two named species were found. Using a combination of characters, testis shape, position of genital pore, and vitelline distribution, a continuum between the two species was established. Again using the above characters a third species, *Helicometrina orientalis* Srivastava, 1936. appears to be an intermediate between *H. nimia* and *H. elongata*. On this basis, evidence will be submitted to place *H. orientalis* and *H. elongata* into synonymy with the type species, *H. nimia*.

33. **Endoparasitic Gastropods of Some Puget Sound Holothurians.** E. S. TIKASINGH, Oregon State College, Corvallis.

During the period July 29 to August 30, 1958, and June 20 to September 10, 1959, 825 holothurians were collected from various regions in Puget Sound, Washington, and examined for endoparasitic gastropods. A total of 13.3 percent of all holothurians examined were infected. However, only 5 of the 11 species of sea cucumbers harbored any parasites. Three species of parasitic gastropods were found in the following holothurians: *Parastichopus californicus* (Stimpson), *Cucumaria lubrica* H. L. Clark, *C. miniata* (Brandt), *Eupentacta quinquesemita* (Selenka), and *E. pseudoquinquesemita* Deichmann. The highest incidences were found in *E. pseudoquinquesemita* (37.8 percent) and *E. quinquesemita* (31.1 percent).

The following holothurians did not have parasitic gastropods: *Cucumaria piperata* (Stimpson), *Pentamera populifera* (Stimpson), *Psolidium bullatum* Ohshima, *Psolus chitonoides* H. L. Clark, *Leptosynapta clarki* Heding, and *Molpadia intermedia* Ludwig.

Thyonicola mortenseni Mandahl-Barth was found in *Cucumaria miniata* and *C. lubrica*, and it is the first record of this genus in American waters. One specimen measured 303.5 cm in length before preservation and it is now the longest parasitic gastropod reported.

A new species of *Thyonicola* was also found from the hosts *Eupentacta quinquesemita* and *E. pseudoquinquesemita*. Further, a new genus of parasitic gastropod was also taken. It was recovered from the sea cucumber *Parastichopus californicus*. Both of these new species will be described elsewhere. (This investigation was supported by a National Science Foundation Training Grant).

34. **Trematode Parasites of the Fish Family Gobiidae from Naples, Italy and Bimini, B.W.I.** D. L. DE GIUSTI AND PIR NASIR, Wayne State University.

Two thousand seven hundred and thirty-two gobiids comprising three species: *Gobius cobitis, Gobius paganellus*, and *Gobius niger jozo*, collected in the vicinity of the Stazione Zoologica, Naples, during the interval of October 1952 through July 1953, were examined for parasites. Trematodes belonging in the following families are present in Gobiidae from Naples: Allocriadiidae, Bathycotylidae, Cryptogonimidae, Hemiuridae, and Monorchiidae.

One thousand and thirty-one gobiids of the genus *Bathygobius* collected from the vicinity of the Lerner Marine Laboratory, Bimini, B.W.I., were examined during December 1955 and May 1960. The trematodes recovered from these fish belong to the families Allocriadiidae, Fellodistomidae, Hemiuridae, Monorchiidae, and Bucephalidae.

Helicometra fasciata of the family Allocriadiidae is present in gobiids of both the Naples and Bimini areas.

35. **Preliminary Report on the Monogenetic Trematodes of Marine Fishes of the Eastern Indo-Pacific.** K. J. KORATHA, University of Southern California, Los Angeles.

A faunistic survey of the monogenes of marine fishes of the eastern Indo-Pacific mainly from the coasts of southern California, Baja California and the Gulf of California is being

completed. Over 1500 fishes belonging to more than 140 species including teleosts and elasmobranchs were examined at different times. Many new species and a few new genera belonging to the following families are recorded: Gyrodactylidae, Dactylogyridae, Calceostomatidae, Udonellidae, Monocotylidae, Capsalidae, Hexabothriidae, Chimaericolidae, Mazocraeidae. Discocotylidae, Microcotylidae, Gastrocotylidae, Diclidophoridae, and Hexostomatidae. The taxonomic descriptions of these species will be appearing in a series of papers dealing with each family.

Of particular interest is the first record of a species of *Calicotyle* and a species of *Calceostoma* from the American coast; these genera were previously reported only from European, Japanese, and Australian waters. There are also new records of species belonging to the recently created sub-families Rhamnocercinae Monaco, Wood, and Mizelle, 1954 and Pterinotrematinae Caballero and Bravo-Hollis, 1955. A very high degree of specificity is noticed in these marine monogenes. The "law of geminate species" or twin species first reported by Jordan (1908) for marine fishes is here extended to the marine monogenes of the amphi-American tropical waters; this phenomenon is monogenes was first observed by the author in 1953 (unpublished) while working on the monogenes of the Gulf of Mexico. It is now confirmed here after studying the present collection. The probable reason for this phenomenon is that the Pacific had direct connection with the Atlantic across the present Central America during long periods of the Paleozoic and Mesozoic eras and in the Cenozoic during the Eocene, Oligocene, Miocene and, according to Schuchert, 1935, even as recent as the lower Pliocene which is only 10 million years ago.

36. **Preliminary Report on the Parasites of a Chimaerid Fish *Hydrolagus colliei* (Lay and Bennett) from the Eastern Indo-Pacific with Comments on the Phylogenetic Significance of Parasitic Distribution in Holocephali.** K. J. KORATHA, University of Southern California, Los Angeles.

Hydrolagus colliei is the only chimaerid found on the Pacific Coast of North America; it ranges from Alaska to northern Baja California. Through the courtesy of the California Fish and Game it was possible to examine two specimens of this fish caught near Cedros Island off the Pacific coast of Baja California. The following parasites were recovered: Monogenea, from the gills, one specimen belonging to the family Chimaericolidae, 21 mm long with a large sac like uterus filled with eggs; Digenea, from body cavity, two adult specimens of *Otodistomum* sp. (Azygiidae), 56 mm long by 9 mm wide; Cestoda, from intestine, four specimens of *Gyrocotyle* sp. (Gyrocotylidae); Hirudinea, from body surface, a single specimen of a piscicolid leech, *Branchellion* sp., 15 mm long with 31 pairs of gills; Copepoda, from gill chamber, three females of a parasitic copepod *Chondracanthus epacthes* Wilson, 1908 (Chondracanthidae). The taxonomy, host specificity and the phylogenetic significance of the distribution of these parasites as well as those previously recorded from Holocephali will be discussed in the light of some "parasitological rules" propounded by Eichler (1948).

37. **Preliminary Report on the Life History of Monogenetic Trematodes with Observations on the Survival of Adults Outside the Host.** K. J. KORATHA AND W. E. MARTIN, University of Southern California, Los Angeles.

The eggs of 15 and the onchomiracidia of 7 species of Monogenea are reported together with their hosts: many are new species. (1) *Udonella caligorum* on a caligid copepod, *Lepeophtheirus parvus*, parasitic on *Pimelometapon pulchrum:* egg single filamented, attached to leg of copepod in clusters; larvae seen emerging, no hooks. (2) *Capsala* sp. on *Sarda lineolata:* egg with four filaments one of which is curved; larva with 12 or more hooks (exact number could not be ascertained). (3) *Entobdella squamula* on *Paralichthys californicus:* egg with single long filament; larva with 14 marginal hooks and three pairs of central anchors. (4) *Benedenia pacifica* on *Myliobatus californicus:* egg with single T-shaped filament; larva with 14 marginal hooks and three pairs of central anchors. (5) *Rhinobatonchocotyle cyclovaginatus* on *Rhinobatos productus:* egg with two filaments; larva with 10 marginal hooks. (6) N. sp. on *Scriphus politus:* egg with single long filament and the coil of another filament visible at its base; larva typical "Microcotyle type." (7) N. sp. on *Paralabrax maculatofasciatus:* egg with two filaments, one short and knobbed, the other long and coiled; larva typical "Microcotyle type." (8) *Spinuris lophosoma* on *Rhinobatos productus:* egg with single filament. (9) Chimaericolid on *Hydrolagus colliei:* egg thin walled with a small protuberance on one end. (10) *Diclybothrium hamulatum* on *Polyodon spathula:* egg without filaments, thin walled and oval. (11) N. sp. on *Sphyraena argentea:* 87 eggs on a single long filament looking like a chain of beads. (12) N. sp. on *Scorpaena guttata:* egg with a single knobbed filament. (13) N. sp. on *Leuresthes tenuis:* two-filamented egg, one short and knobbed, the other long and coiled. (14) N. sp. on *Menticirrhus undulatus:* one-filamented egg whose base is coiled up like a spring. (15) *Heteraxine meservei* on *Seriola dorsalis:* egg with long, single filament.

Kept at a temperature of − 5 C, *Udonella caligorum* survived for 4 days (fixed while alive); *Microcotyle* sp., *Hexostoma* sp. and a hexabothrid lived for a week; *Entobdella squamula* lived more than 18 days but decreased in size. (This work is being supported by a grant from the National Science Foundation.)

38. **Trematodes from Shorebirds Collected at Morro Bay, California.** H. T. RUSSELL, JR., University of California, Los Angeles. (Present address: University of California, Santa Barbara).

Trematodes assignable to 10 families and 26 species (8 species apparently undescribed) were recovered from 110 shorebirds of 8 species. Three species of Philophthalmidae, five species of Echinostomatidae, seven species of Microphallidae, two species of Gymnophallinae, two species of Galactosomatidae and one species of Notocotylidae were recovered from the intestines and caeca. Two species of Eucotylidae and one species of Renicolinae were from the kidney. Two species of Cyclocoelidae were from the body cavity. At least one species of Schistosomatidae was associated with the intestine and liver.

Host specificity varied. The philophthalmids and one of the gymnophallines were the least host specific and the echinostomes were the most host-specific.

No absolute host-sex specificity was found. Male hosts contained more adult philophthalmids than female hosts. For six of the species of microphallids, more males than females were infected and males contained more worms.

October collections had more juvenile than adult worms; February collections had more adults than juveniles; and November collections were intermediate between these two conditions. According to these adult to juvenile ratios, it appears that the shorebirds were infested by many of the worms along their southern migration route. The presence of juveniles in all collections, including two killed while leaving the metacercarial cyst, would indicate infestations in the vicinity of Morro Bay.

39. **Life History of *Himasthla* sp., an Echinostome Trematode.** J. E. ADAMS AND W. E. MARTIN, University of Southern California, Los Angeles.

The common mudflat snail, *Cerithedea californica*, collected at Newport Bay, California, is naturally infected with the cercariae of this species. The cercariae were allowed to encyst on the bottom of glass finger bowls. Gravid adults were obtained from the intestine of hatchery-raised chicks 17 or more days after feeding the metacercariae. Eggs from these adults were incubated in sea water at room temperature, and miracidia emerged after 18 days. Members of Himasthlinae are normally parasitic in the intestine of birds.

40. **The Life History of *Echinochasmus milvi* Yamaguti, 1939.** J. E. MCCAULEY AND I. PRATT, Oregon State College, Corvallis, Oregon.

A previously undescribed zygocercous or "rattenkönig" cercaria found in *Oxytrema silicula* in Oregon and Washington was found to encyst loosely in the gills of sticklebacks and guppies, experimentally, and develop into mature *Echinochasmus milvi* in the hamster and the duck. This is the first time that a zygocercous cercaria has been implicated in a life cycle.

41. *Deroceras laeve*, **a New First Intermediate Host of *Panopistus pricei* Sinitsin, 1931 (Trematoda: Brachylaimidae).** J. B. VILLELLA, Phoenix Memorial Laboratory, University of Michigan.

Deroceras laeve, a land slug, is a new and the fourth reported first intermediate host of *Panopistus pricei*, a brachylaimid from the small intestine of the short-tailed shrew *Blarina brevicauda*.

Of 177 slugs examined for larval trematodes during the late fall of 1959, 1 slug was shedding unidentified brevicaudate cercariae. These cercariae were transferred to two laboratory-raised snails, *Zonitoides nitidus* and *Ventridens ligera*. In 70 to 80 days mature metacercariae were recovered from the heart and pericardial chamber of these snail hosts. A study of morphologic characters and the terminal position of the genital pore suggested that this metacercaria belonged to the genus *Panopistus* Sinitsin, 1931.

Previous studies on *P. pricei* by Villella (1954, J. Parasit. **40**: 470–472) disclosed that this metacercaria did not develop in several experimental hosts tested. Consequently, eggs of mature flukes obtained from the short-tailed shrew were fed to laboratory-raised slugs. In 37 to 50 days brevicaudate cercariae from branching sporocysts were shed from the slugs. These cercariae subsequently produced metacercariae identical to the metacercariae from the naturally infected slug. Morphologic study and measurements of the larval stages from *D. laeve* agree essentially with data on the larval stages of the previously reported first intermediate hosts of *P. pricei*, viz. *Zonitoides arboreus*, *Agriolimax agrestis* and *Ventridens ligera*.

42. **On the Infection of Birds and Mammals with the Cercaria and Metacercaria of the Eye-Fluke,** *Philophthalmus.* J. E. ALICATA AND H. L. CHING, University of Hawaii, Honolulu.

Groups of young chickens, rabbits and rats (three in each group) have been exposed to infection with cercariae or excysted metacercariae of *Philophthalmus* sp. derived from naturally infected snails, *Tarebia granifera mauiensis.* The excysted metacercariae were obtained by exposing the encysted forms to warm water. With the aid of a pipette the larval flukes were withdrawn from the water and quickly transferred to the eye or back of the mouth of the experimental animals as follows: Groups I (rabbits) and II (chicks), 25 cercariae in the eye; groups III (rabbits) and IV (chicks), 100 cercariae by mouth; groups V (rabbits) and VI (chicks), 25 metacercariae in the eye; groups VII (rabbits), VIII (chicks), and IX (white rats), 100 metacercariae by mouth. Twenty-six days after infection the following number of mature or nearly mature flukes were recovered from the eyes of each of the three animals from each group: Group I, 0, 3, 0; group II, 4, 4, 13; group III, 0, 0, 0; group IV, 7, 16, 8; group V, 1, 2, 4; group VI, 22, 19, 21; group VII, 0, 0, 0; group VIII, 22, 30, 24; group IX, 0, 0, 0.

It is concluded that birds and mammals can be infected with cercariae and metacercariae of *Philophthalmus* by the ocular route. Birds can also become infected with cercariae and metacercariae by the oral route. These results indicate possible methods of human infection as reported in the literature. (This study was supported by a U. S. Public Health Service Grant No. E-1647-C2).

43. *Bulinus (Bulinus) forskalii* **Ehrenberg, 1831: Intermediate Host of** *Gastrodiscus aegyptiacus* **(Cobbold, 1876) Looss, 1896.** E. A. MALEK, Tulane Medical School.

Looss (1896) claimed that in Egypt the operculate snails *Cleopatra bulimoides* and *C. cyclostomoides* were the hosts of *G. aegyptiacus* because of the similarity of amphistome cercariae from these snails to the adult worms. These hosts have since been quoted in many textbooks. Le Roux (1958) experimentally infected *Bulinus (Bulinus) forskalii* and *B. (B.) senegalensis* in London. *B. (B.) forskalii* naturally infected with amphistomes were collected from a swampy area in the White Nile reservoir, Sudan, frequented by horses and donkeys heavily infected with *G. aegyptiacus.* Operculate snails from the same area, *Lanistes carinatus, Pila wernei,* and *Gabbia senaariensis,* were negative for amphistomes. When 1-week-old metacercarial cysts from *B. (B.) forskalii* were fed to a 2-month-old donkey, eggs of *G. aegyptiacus* were recovered from the feces 155 days later and adult worms at autopsy at 162 days. The donkey was in another part of the country where infection with this fluke is rare and light, did not harbor the fluke, was kept after infection in a stall in a veterinary clinic and fed alfalfa from a farm where the canals were thoroughly treated with molluscicides for another project. The donkey was simultaneously exposed to cercariae of *Schistosoma bovis* and readily became infected. It is very likely that *B. (B.) forskalii* is not the only host for *G. aegyptiacus.*

44. **The Florida Spotted Gar, as the Intermediate Host for** *Odhneriotrema incommodum* **(Leidy, 1856) from** *Alligator mississippiensis.* W. H. LEIGH, University of Miami.

Odhneriotrema incommodum (Leidy, 1856) was described from the mouth of *Alligator mississippiensis.* Since that time it has been restudied by Canavan (1933) and McIntosh (1935). No reference to the life history of this large member of the family Clinostomidae occurs in the literature. Large, orange capsules, occurring primarily in the ovaries of the gar, *Lepistosteus platyrhincus,* have been fed to five *A. mississippiensis* and infection occurred in all individuals. After release from the fibrous capsule, the trematode migrates anteriorally and its initial attachment occurs at the junction of esophagus and pharynx with later migration to the nasopharynx or area adjacent to the glottis.

Adults apparently derive their nourishment from tissue juices and blood by burying their conical anterior ends in the tissues. After a time they move, leaving hard, conical fibrous pits of host origin that appear to separate them from their food supply. Worms become sexually mature within 80 days but retain their embryonated eggs until there is contact with water. Within 3 minutes after contact with water, excised specimens shed their eggs explosively in a long chain tenuously held together by a mucoid secretion. Eggs hatch almost simultaneously within 2 to 3 minutes after deposition.

Collection data indicate that there is an unusual and unexplained affinity of the metacercaria for female gar. While the majority occur in the ovary, smaller numbers occur in mesenteries and fatty tissues. Of the 253 female gar examined, 93 (37 percent) were infected; of the 214 male gar checked only 18 (8 percent) were infected. Of the 227 metacercariae collected, 199 (88 percent) occurred in female gar and only 24 (12 percent) were recovered from the testes of male gar.

45. **Experimental Infection of Sheep with the Dog Ascarid,** *Toxocara canis.* W. F. SCHAEFFLER, University of Illinois.

The ubiquity of *Toxocara canis,* the common roundworm of dogs; and the frequent association of sheep with dogs suggested the possibility that migrating *Toxocara canis* larvae might well be a hitherto unrecognized cause of injury to sheep. Experiments were therefore conducted to determine the migratory route and pathogenesis of *Toxocara canis* in sheep and to delineate the host spectrum of this parasite in a previously uninvestigated host.

Eight yearling sheep and 10 lambs, 2 to 10 days old, were fed 4000 to 216,000 infective *T. canis* eggs. They were killed after 2 days to 12 weeks and examined for gross and microscopic lesions. Their mesenteric lymph nodes, pancreas, liver, lungs, heart, kidneys, brain, and skeletal muscles were examined for larvae by digestion with artificial gastric juice and by allowing the larvae to migrate into physiological salt solution. Total erythrocyte, total white blood cell and differential white blood cell counts and hemoglobin determinations were made before exposure and at intervals thereafter.

The only significant blood change following the infection was a high eosinophilia (22 percent). The migratory pattern of the *T. canis* larvae was definitely influenced by the age of the hosts. In the yearlings, hardly any migration took place beyond the liver. In the very young lambs the mesenteric lymph nodes, pancreas, heart, lungs, kidneys, brain, and the shoulder and thigh muscles were invaded as early as 4 days after infection.

The larvae remained alive without apparent diminution in numbers for the entire observation period (12 weeks in the yearlings and 8 weeks in the lambs). They remained in the second stage and did not increase in size. The average length of 225 larvae was 425 microns and their average width 22 microns.

No clinical signs due to the infection with *T. canis* larvae were observed. Obvious pathologic changes occurred in the intestinal lymph nodes, liver, and lungs. The lesions consisted of hemorrhages, infiltration with mononuclear cells, neutrophiles and especially with eosinophiles, and focal necrosis. However, there were hardly any cellular changes around the actively migrating larvae in the brain and other organs.

46. **On the First Intermediate Host of** *Paragonimus ohirai* **Miyazaki, 1939 and** *P. iloktsuenensis* **Chen, 1940.** I. MIYAZAKI, Kyushu University, AND Y. YOSHIDA, Kyoto Prefectural University of Medicine, Japan.

It is demonstrated experimentally that both species of *Paragonimus* can complete development in four species of snail hosts: *P. ohirai* developed in 51 of 82 *Assiminea parasitologica* (62.2 percent) exposed, in 19 of 33 *A. yoshidayukioi* (57.6 percent), in 1 of 50 *A. japonica* (2 percent), and in 12 of 40 *Paludinella japonica* (30 percent), while *P. iloktsuenensis* developed in 17 of 30 *A. parasitologica* (56.6 percent), 17 of 26 *A. yoshidayukioi* (65.3 percent), in 2 of 78 *A. japonica* (2.6 percent), and in 1 of 12 *P. japonica* (8.3 percent).

Eight of 17,942 *A. parasitologica* (0.045 percent) and 1 of 412 *A. yoshidayukioi* (0.24 percent) collected in Hyogo Prefecture were found to be naturally infected with larval *P. ohirai,* but 5,118 *A. japonica* from this Prefecture were uniformly negative for this species of *Paragonimus.* Of 2,120 *A. parasitologica,* collected in Osaka City, 7, or 0.33 percent, were naturally infected with *P. iloktsuenensis.* The natural incidence of the other snail hosts with this species of *Paragonimus* is not yet known.

It thus appears that in Japan the first intermediate hosts of *P. ohirai* are *A. parasitologica* and *A. yoshidayukioi,* while that of *P. iloktsuenensis* is *A. parasitologica.* The newly discovered host, *A. yoshidayukioi,* is very small (about 3 by 2 mm). It was discovered by the junior author and described by Kuroda (1959, Venus **20:** 335–338).

The cercariae of both species of *Paragonimus* resemble one another in shape, but *P. ohirai* has 20 terminal cells while 60 of these are present in *P. iloktsuenensis.*

47. **A New Nematotaeniid from the Salamander,** *Plethodon vandykei,* **from Northern Idaho.** J. A. WAITZ AND K. N. MEHRA, University of Illinois.

Several specimens of small Nematotaeniids were recovered from the intestinal tracts of adult Couer d'Alene salamanders, *Plethodon vandykei idahoensis,* captured at Wolf Lodge Bay, Lake Couer d'Alene, Kootenai Co., Idaho. The specimens were removed from the gut and fixed in A.F.A. Following fixation the specimens were kept in 70 percent ethanol until stained in either Semichon's acetic carmine or Ehrlich's Haematoxylin. Sections were made of paraffin imbedded specimens and these were stained with Harris' Haematoxylin.

After examination, the specimens were assigned to the genus *Baerietta* Hsü, 1935. They differ from the five existing species of the genus in the diameter, size of suckers, constriction of the neck region, size of the uterus, and number of eggs.

Cross sections of mature and gravid proglottids as well as a whole mount demonstrating filling of paruterine organs are shown. (This investigation was supported in part by a grant from the Northwest Scientific Association and was completed under the tenure of a fellowship (EF-9587) from the National Institute of Allergy and Infectious Diseases, U. S. Public Health Service.)

48. **Passeriform Birds as Experimental Hosts for *Posthodiplostomum minimum* (Trematoda: Diplostomatidae).** M. J. ULMER, Iowa State University and Iowa Lakeside Laboratory.

During the summers of 1959 and 1960 at the Iowa Lakeside Laboratory at Lake Okoboji, several experiments were conducted, involving the feeding of metacercariae of *P. minimum* from the livers of naturally infected pumpkinseeds (*Lepomis gibbosus*) to several species of passeriform birds, namely two robins (*Turdus migratorius*), four red-winged blackbirds (*Agelaius phoeniceus*), and two yellow-headed blackbirds (*Xanthocephalus xanthocephalus*).

Results of these experiments indicate that the robin serves as a suitable definitive host for these diplostomatid flukes which in nature parasitize birds of the family Ardeidae. In one young robin fed June 25, 1959, 182 worms were recovered 40 hours post-feeding. Whole mounts of 97 showed that 47 of them (48 percent) were gravid. In June 1960, a second young robin, 48 hours post-feeding, harbored 240 gravid adults and 22 immature ones. Gravid worms from the robin are identical to those collected from herons of the Okoboji region.

Experiments involving young red-winged blackbirds resulted in the failure of metacercariae to attain sexual maturity. In one experiment carried on in June 1960, 144 immature worms were recovered 40 hours post-feeding. In a second experiment, only two immature worms were recovered 40 hours post-feeding. Two additional experiments involving the use of red-wings were negative 48 hours after the birds were fed.

Two yellow-headed blackbirds were also negative for these parasites 48 hours after having been fed metacercariae. (This investigation was supported in part by a grant from the National Science Foundation.)

49. **The Life Cycle of *Marsipometra hastata* (Linton, 1898) Cooper, 1917.** F. P. MEYER, Iowa State University.

Various stages in the life history of *Marsipometra hastata* (Linton, 1898) Cooper, 1917, a cestode parasite of the paddlefish, *Polyodon spathula* (Walbaum, 1792) Refinesque, 1820, have been reared in the laboratory. Gravid worms release large numbers of eggs which hatch upon contact with fresh water warmer than 60 F. Lower temperatures greatly retard hatching. Coracidia of *M. hastata* closely resemble those of other pseudophyllideans. Their activity and life span are directly affected by water temperatures, and below 55 F. the larvae are inactive. *Cyclops bicuspidatus* Claus serves as an intermediate host for *M. hastata*. Onchospheres penetrate the wall of the intestine within six hours after their ingestion by the copepod. Early development within the haemocoel is rapid and within 2 weeks, small plerocercoids are produced. Normal procercoid stages are not formed and the larvae do not develop a cercomer. Stages recovered from the haemocoel of *G. bicuspidatus* approximate the size and shape of the smallest worms recovered from the intestines of naturally-infected paddlefish. Feeding experiments to complete the life cycle have been impossible because of the unavailability of parasite-free *Polyodon*, but evidence is presented suggesting that a second intermediate host is not required.

A study of the morphology of the three previously described species of *Marsipometra* was undertaken. Of these, *M. parva* is accepted as a valid and separate species. Careful comparisons of type specimens of *M. confusa* and *M. hastata* with material collected in this study suggest the feasibility of reducing *M. confusa* to synonymy with *M. hastata*.

50. **Microfilarial Periodicity of *Splendidofilaria quiscali* (von Linstow, 1904) n. comb. (Nematoda: Onchocercidae).** J. A. ODETOYINBO AND M. J. ULMER, Iowa State University, Ames.

Splendidofilaria quiscali (von Linstow, 1904) n. comb., a nematode inhabiting the cerebral hemispheres of bronzed grackles (*Quiscalus quiscula versicolor*), produces sheathed microfilariae whose periodicity has been very intensively studied. Experiments have been performed on normal periodicity, reversal of periodicity, effect of continuous light and continuous darkness on periodicity, and on the re-establishment of normal periodicity following reversal. These experiments involved withdrawal of 0.0125 ml blood from metatarsal veins of six infected birds every 2 hours for 48 to 72 consecutive hours.

Microfilaria quiscali exhibits well-marked nocturnal periodicity with maximum numbers generally occurring around midnight. When infected birds are supplied with artificial light be-

tween 8 p.m. and 8 a.m., periodicity is reversed, there being more microfilariae in films made between 8 a.m. and 8 p.m., when birds were in darkness. When infected birds were exposed to continuous light for 5 consecutive days, and periodicity experiments conducted during the last 2, normal nocturnal periodicity was disrupted in all but one, in which an apparently regular maximum number of microfilariae occurred about 6 p.m. However, maximum numbers of microfilariae far exceeded those in earlier experiments concerned with normal and reversed periodicity. Normal nocturnal periodicity was re-established when those birds which had been kept under continuous light were maintained for 5 days under normal conditions of light and darkness. However, maximum numbers of microfilariae in peripheral circulation were reduced considerably. When these same birds were then kept in the dark for 5 consecutive days, microfilariae maintained their normal nocturnal periodicity.

51. **A Hemagglutination Test for Entamoebae.** J. F. KESSEL, W. P. LEWIS AND S. MA, The University of California, Los Angeles, and the Estelle Doheny Eye Foundation, Los Angeles.

Agglutinins produced in rabbits, by injecting antigens from different species of *Entamoeba*, have been demonstrated in hemagglutination tests employing antigens adsorbed to tanned human O cells.

Antigen of *E. histolytica*, prepared from cultures grown with *Trypanosoma cruzi*, has likewise been used successfully in a similar hemagglutination test with sera from known cases of amebiasis. Procedures and results of these tests will be demonstrated.

52. **Observations on the Ultrastructure and Histochemistry of Cercarial Glands.** F. J. KRUIDENIER, University of Illinois.

Several investigators have differentiated the pre- and postacetabular glands of *Schistosoma mansoni* and special glands of xiphidiocercariae on the basis of their histochemical reactivity. Present studes correlate the ultrastructure of these glands with previously noted histochemical reactions. They extend the studies to subcuticular glands of *S. mansoni* and an armate xiphidiocercaria, tentatively identified as *Tetrapapillatrema concavocorpa*, or a closely related species.

The ultrastructure of the glands of *S. mansoni* was determined in specimens from experimentally infected *Australorbis glabratus*. The armate cercaria was obtained from naturally infected *Helisoma trivolvis*. Normally emerged cercariae were fixed in the buffered osmium solution of Palade at pH 8.6 and embedded in methacrylate for study with the RCA-EMU-3C electron microscope. Similar Bouin-fixed specimens and aliquots of the osmium-fixed specimens were used for the histochemical tests.

Preacetabular, postacetabular, and the few subcuticular glands present in *S. mansoni* can be differentiated by their ultrastructure. Series of Schiff-positive subcuticular cystogenous glands are present in the armate cercaria. These appear to be histochemically similar to glands observed in other, diverse species of cercariae. The ultrastructure of the glands of the armate species also differentiates the subcuticular, cystogenous glands. (This investigation was supported in part by a research grant (E-1319) from the National Institutes of Health, Public Health Service.)

53. **The Accessory Piece in the Differentiation of Certain Oxyurids of the Genus *Syphacia* Seurat, 1916.** F. J. KRUIDENIER AND K. N. MEHRA, University of Illinois.

Type specimens of *Syphacia peromysci* Harkema, 1936, are not available. The type locality for the species is Durham County (Duke Forest) North Carolina. The type host is *Peromyscus l. leucopus* Thomas, 1895. Specimens for the present study were obtained from *P. l. leucopus* trapped in Wake County, N. C., adjacent to Durham County. Temporary preparations of specimens cleared in glycerine and permanent preparations of mature males mounted in glycerine jelly were studied. Special attention was paid to characteristics which would differentiate the males of this species from those of other closely related species. A Leitz Ortholux microscope with a 100 × oil immersion objective and 15 × oculars was used for the study of the accessory piece.

The accessory piece of *S. peromysci* is attached to the distal end of the gubernaculum. It is sharply pointed distally, bulbous proximally. It is recurved, lengthwise, into the shape of an attenuate S. The pointed end commonly protrudes from the anus of our specimens. More or less prominent muscle fibers attach to the proximal end. The ventrolateral surfaces of the accessory piece possess five, bilateral pairs, of blunted, minute projections (three pairs in one of 25 specimens). These appear similar to the minute irregularities described previously for *S. thompsoni* Price, 1928 (from *Sciurus carolinensis*).

The accessory pieces of neither *S. obvelata* (Rudolphi, 1902) nor of *S. muris* (Yamaguti, 1941) possess the minute projections described herein. They are, therefore, useful criteria in the differentiation of *S. peromysci* from other, similar species of these small oxyurid nematodes.

(This investigation was supported in part by a research grant (E-1319) from the National Institute of Health, Public Health Service.)

54. **The Internal Anatomy of the Female Spiny Rat Mite,** *Echinolaelaps echidninus.* L. A. JAKEMAN, U. C. L. A., AND R. W. STRANDTMANN, Texas Technological College.

The digestive system of *Echinolaelaps echidninus* consists primarily of a small ventriculus with its three pairs of tube-like caeca. Two pairs of globose cells or glands, not previously described, are found in the alimentary canal. The paired excretory or malpihgian tubules extend the length of the body and empty into the hind gut or rectum. One pair of coxal glands is found above and median to the coxae of legs I. No true circulatory system exists in *E. echidninus;* there is no heart as is present in some of the other Mesostigmata. The nervous system consists of a central brain mass surrounding the esophagus and paired nerves serving the various body parts. A single median nerve serves the epipharynx. A vacuolated area found medial to the palpal nerves is described. This structure is possibly glandular in function. Cells which are probably sensory in nature occur in the epipharynx and tectum. The respiratory system consists of paired stigmal openings posterior to legs III, atrial chambers, and tracheae going to all parts of the body. The reproductive system contains a globular ovary, oviduct, and vagina as well as structures found only in gamasid mites including lyrate organ, sacculus foemineus, and ringed tubes. Paired vaginal glands are present.

55. **Method of Culturing Psocids for Use in Parasitological Investigations.** R. W. ALLEN, Animal Disease and Parasite Research Division, A. R. S., U. S. D. A., University Park, New Mexico.

The discovery by the writer (1959, J. Parasit. **45:** 537–538) that embryos of the fringed tapeworm, *Thysanosoma actinioides,* of sheep undergo larval development in insects of the order Corrodentia or "book lice" suggests that further investigation of these insects as possible intermediate hosts of other parasites might be fruitful. Since the procedures described in the literature for rearing these insects are generally not suited to parasitological investigations, the method used by the writer may be of interest to other parasitologists engaged in similar investigations.

The culture chambers were small stender dishes lined on the bottom and lower half of the sides with a mixture of plaster of paris and activated carbon, a substrate suggested by Dr. G. W. Wharton, head of the Zoology Department, University of Maryland. Lids were made of square pieces of plate glass with holes 1 cm in diameter in the center. The holes were covered with 200-mesh screen held in place with cellophane tape. The psocids could be easily transferred from one of these chambers to another, and parasite eggs and food could be added to the cultures without difficulty. By substituting a solid piece of plate glass for the ventilated one, the insects could be readily observed and CO_2, which was used to control these very active insects before microscopic examination, could be administered.

Constant temperatures of 25 to 26 C. and relative humidities of 70 to 76 percent were satisfactory for culturing the species collected to date from New Mexico. Rolled whole wheat, Pablum, and/or dry active yeast were suitable foods. (This work was carried out in cooperation with the New Mexico Agricultural Experiment Station.)

56. **Observations on the Structure of Cysticercoids.** M. VOGE, University of California, Los Angeles. (See abstract #24).

57. **An Electron Microscopic Study of** *Entamoeba histolytica.* J. H. MILLER AND J. C. SWARTZWELDER, Louisiana State University, New Orleans.

Trophozoites of *Entamoeba histolytica* (strain NRS) from 48-hour cultures on modified Boeck-Drbohlav media were fixed in various solutions, including 1 percent osmium tetroxide, 3 percent chromic oxide with 4 percent formalin, 3 percent chromic oxide and 0.6 percent potassium permanganate. The organisms were embedded in methacrylate, sectioned and examined in an RCA EMU-3 at 50 KV.

The endoplasmic reticulum of the amoeba is composed of a number of scattered circular and, less often, oval profiles. Potassium permanganate fixation demonstrates a density difference between endoplasm and ectoplasm. A nuclear pore system was demonstrated after osmium fixation. Granular aggregates located in the chromatin material, but limited to small areas of the nucleus, were seen with all fixatives. These correspond to the "differentiated areas" of *E. invadens* (Deutsch and Zaman, 1959, Exp. Cell Res. **17:** 310–319) and the "chromatin granules" of *E. histolytica* (Osada, 1959, Keio J. Med. **8:** 99–108).

No evidence of Golgi apparati or mitochondria was found.

Electron micrographs in the exhibit depict the fine structure of *E. histolytica.* (This study supported by Grants E-2347 and H-2549, National Institutes of Health.)

58. **Changes in Schistosome Larvae and in Mouse Host Skin During Migration of the Parasites.** M. A. STIREWALT, Naval Medical Research Institute.

Development of schistosomula in skin will be presented in four stages against the background of the histology of cercariae in the snail host. Loss of acetabular gland contents, enlargement of the gut sacs, and an increase in numbers and types of cells will be demonstrated. Reactions of skin tissues to these invading schistosomes will be presented in four stages. They consist of early thickening of the stratum corneum and its infiltration by host neutrophils, necrosis of host epidermal cells, hemorrhage, and finally the aggregation in invaded dermal areas of large numbers of eosinophils, lymphocytes and fibrocytes or mast cells.

59. **Studies on the Genus *Metorchis* Looss, 1899 (Trematoda: Opisthorchiidae).** F. J. ETGES AND M. L. SHARMA, University of Cincinnati.

More than 25 adult specimens of *Metorchis*, collected by Dr. B. B. Babero of Southern University, Baton Rouge, La., provide data which indicate the existence of a species closely related to *Metorchis albidus* (Braun, 1893). About 500 worms were taken from the biliary ducts of an Alaskan husky dog in Kotzebue, Alaska, in 1950. Babero and Rausch (1952, Proc. Helm. Soc. Wash. **19:** 15–17) considered these specimens insufficiently distinct from *M. albidus* to justify a new species.

The present study shows that among 14 species of the genus *Metorchis*, Babero's specimens are most similar to *M. albidus*, but that the following constant differences exist: Body size up to twice that of *M. albidus*, with most organs correspondingly larger; ovary and oral sucker of smaller size than *M. albidus;* position of ovary not anterior to seminal receptacle as in *M. albidus*, but always dorsal to it. The range of variation in *M. albidus* given by K. I. Skrjabin (1950, Trematodes of Man and Animals. Vol. IV.) does not include those found in the present specimens. It is further pointed out that the host of *M. albidus* is the same as that of the present material, hence the differences in structure and size are not likely due to the factor of host difference.

60. **Trematode Parasites of the Fish Family Gobiidae from Naples, Italy, and Bimini B. W. I.** D. L. DE GIUSTI AND P. NASIR, Wayne State University. (See abstract #34.)

61. **The Development of *Trichinella spiralis* in the Dormouse, *Eliomys quercinus*.** R. M. CHUTE, San Fernando Valley State College.

Eight adult dormice were infected with 550 trichina larvae each. The larvae were recovered from an experimentally infected white rat. Larvae from the same rat were administered to a litter of six white rats, 6 weeks of age. Twenty-three adult trichina were recovered from the intestine on a dormouse examined on the fifth day of the infection. The worms were distributed throughout the length of the intestine. Microscopic examination of the 15 female trichina recovered from this host revealed no vermiform embryos although eggs were present. Four adult female trichina, all containing vermiform embryos, were recovered from a dormouse examined on the 20th day of the infection. Twelve uncoiled larvae were seen upon microscopic examination of diaphragm of this host. An average of 1,920 larvae were recovered from six dormice on the 31st day of infection. Individual recoveries ranged from 5 to 2,500. An average of 38,950 larvae were recovered from six white rats with individual recoveries ranging from 10,350 to 70,650. No adult trichina were recovered from the intestine of either dormice or rats on the 31st day after infection.

62. **The Influence of Hibernation on the Development of *Trichinella spiralis* in the Dormouse, *Eliomys qercinus*.** R. M. CHUTE, San Fernando Valley State College.

Eighteen adult dormice were infected with 550 trichina larvae each. A similar number of larvae, recovered from the same experimentally infected rat, were administered to six white rats 6 weeks of age. Twenty-four hours after infection, six infected dormice were placed at 6 C. Individual animals entered hibernation 36 to 48 hours after infection. All six were removed to room temperature 72 hours after infection after experiencing 24 to 36 hours of hibernation. A second group of six infected dormice were placed at 6 C on the 15th day after infection and removed to room temperature 94 hours after initial exposure to cold. Individual animals entered hibernation 10 to 60 hours after exposure to cold and periods of hibernation varied from 36 to 84 hours. A third group of six infected dormice were held at room temperature as were the six infected rats. Thirty days after infection all animals were killed and trichina larvae recovered by artificial digestion. The six rats yielded from 10,350 to 70,650 larvae (mean 38,950); the dormice hibernating on the third to fourth day after infection yielded 0 to 600 larvae (mean 125); the dormice hibernating between the 15th and 17th day of infection

yielded 50 to 2,650 larvae (mean 1,660); the non-hibernating dormice yielded 5 to 2,250 larvae (mean 1,920). There was no relation evident between individual worm recoveries and length of hibernation in either group of dormice exposed to cold. On two occasions dormice in the group held at room temperature were observed in topor with body temperatures between 24 and 28 C. There was no relation between these depressions of body temperature and worm recoveries.

63. **Sylvatic Trichinosis in Carnivorous Mammals in the Rocky Mountain Region of Colorado.** O. W. OLSEN, Colorado State University.

With the knowledge that trichina may be transmitted through feces resulting from meals of trichinous flesh, the role of sylvatic trichinosis in wild animals assumes greater importance in relationship to its transmisson to humans.

Investigations on the prevalence of infections of trichina in eight species of predatory mammals in the Rocky Mountain region of Colorado revealed a relatively low incidence of the parasite. From a total of 645 tongues examined, including 394 bobcats (*Lynx rufus*), 193 coyotes (*Canis latrans*), 33 foxes (probably all *Vulpes fulva*), 11 skunks (probably all *Mephites mephites*), 6 mountain lions (*Felis concolor*), 5 badgers (*Taxidea taxis*), 2 raccoons (*Procyon lotor*), and 1 bear (*Ursus americanus*) supplied by personnel of the U. S. Fish and Wildlife Service, Predator Control Branch, six animals were infected. They included 4 bobcats (1 percent), 1 coyote (0.5 percent), and 1 fox (3 percent). The higher incidence in foxes may be due to their propensity to associate more closely with human habitations than either the coyotes or bobcats.

As yet, too little is known in relation to how effectively *Trichina* utilizes the total food chain in a population of invertebrates and vertebrates to evaluate the picture of sylvatic trichinosis. (Supported in part by Research Grant E-976(C-3), National Institutes of Health, U. S. Public Health Service.)

64. **Attempts to Modify Innate Resistance of Chinese Hamsters to** *Trichinella spiralis.* A. L. RITTERSON, The University of Rochester, School of Medicine and Dentistry.

Chinese hamsters exhibit a pronounced innate resistance to the muscle phase of *T. spiralis* while refractoriness to the intestinal phase seems unremarkable (Ritterson, 1959, J. Inf. Dis. **105**: 253).

Although cortisone abolishes resistance (loc. cit), the mechanisms remain obscure. Attempts to ameliorate resistance by other means should help to clarify the nature of host response as well as the mode of action of cortisone.

Administration of ACTH (0.5 unit twice daily) exhibited no effect on innate resistance of muscle tissue to *T. spiralis* even though the hormone suppressed lymphoid tissue response as measured by spleen weight differences in treated and untreated, infected hamsters. Similarly, adrenalectomy did not alter host resistance.

Methyl testosterone at dose levels of 0.6 mg per day or more resulted in a small but measurable increase in carcass larva yields after 35 days of infection. Since the inflammatory response was not grossly affected by the steroid, it is possible that its action is related to its anabolic effect on muscle tissue.

6-Mercaptopurine (0.1 and 0.2 mg per day) was employed because of its inhibitory effect on antibody production. Infection with *Leishmania donovani* was used as an R-E cell blocking agent. Neither of these methods altered the capacity of Chinese hamsters to reject the muscle phase of *T. spiralis*. Similarly, infective doses of *T. spiralis* as high as 1200 larvae did not overcome tissue resistance. The latter dose was found to be uniformly fatal to golden hamsters.

Concentrated extracts of golden hamster carcass were administered daily (i.p.) to trichina-infected Chinese hamsters and were not found to influence host resistance. Chinese hamster carcass extracts did not measurably inhibit the establishment of muscle larvae in golden hamsters. Forssman antibody was not found in Chinese hamster serum. Forssman antibody was produced in rabbits suitably treated with carcass extracts of Chinese hamsters.

Chinese hamsters on a prolonged regimen of a diet containing a high level (2 percent) of cod liver oil showed a significant ameliorization of innate resistance to *T. spiralis*.

65. **Immunological Tolerance Studies with White Mice and** *Trichinella spiralis.* L. J. OLSEN AND A. EWERT, The University of Texas Medical Branch.

Previous studies from this laboratory have been reported in which intracardiac injections of metabolic (excretions and secretions of cultured larvae) or somatic (sonicated larvae) antigens were given to newborn mice (Fairfield-Webster) in attempts to induce host tolerance to a subsequent sublethal challenge with living larvae. Data will be presented summarizing

recent experiments designed to induce tolerance with this system by transabdominal intrafetal injection of metabolic antigens or subcutaneous injection of living larvae in newborn mice with host response measured by the use of sublethal or LD_{50} challenge with living larvae. (These studies were supported by National Science Foundation Research Grant, G-7104.)

66. **Resistance to *Trichinella spiralis* following infection by *Nippostrongylus muris*.** C. D. LOUCH, The Johns Hopkins University.

The purpose of this study was to determine whether infection of rats with *Nippostrongylus muris* would give protection against subsequent infections with *Trichinella spiralis*.

Thirteen male white rats of the same age were infected with 1200, 4600, and 8000 *N. muris* larvae at 5-day intervals. Fourteen days after the last infection blood was taken from three of these rats for agar-diffusion tests with a saline extract of *T. spiralis* larvae. The control consisted of 10 male rats not infected with *N. muris*.

The animals of both the control and experimental groups were infected intragastrically, 14 days after the last *N. muris* infection, with 1000 *T. spiralis* larvae each. Counts of the *T. spiralis* larvae were made 30 days later using the method of Larsh and Kent (1949, J. Parasit. **35**: 45–53).

The mean larval count for the experimental group was significantly lower than that for the control group as indicated by Student's *t* test. This would indicate that previous infection with *N. muris* protected the rats to some extent against *T. spiralis*. The agar-diffusion tests showed no reaction between serum of *N. muris* infected rats and *T. spiralis* antigen. (This study was supported by Grant number EF-9311 from the National Institutes of Health.)

67. **Extraction of Immunologically Active Protein Complexes from *Trichinella spiralis* Larvae.** P. F. DIRK VAN PEENEN AND H. N. KENT, The Johns Hopkins University, School of Hygiene and Public Health.

The nature and composition of crude larval extracts commonly used in serological tests for trichinosis are not known. Consequently, it is difficult to consider as specific the immunological and serological reactions taking place with such ill-defined mixtures. The need for isolated and chemically identified specific antigens is apparent. The present report is concerned with the extraction and identification of such antigens from *Trichinella spiralis* larvae.

Fresh *Trichinella spiralis* larvae, obtained by digestion of heavily infected rat carcasses by pepsin and HCl, were delipidated by absolute ethanol and anhydrous ether successively, at very low temperature (−70 C). The delipidated powder was then extracted exhaustively, first with triple distilled water, then with a 0.85 percent NaCl solution, and finally with a 10 percent NaCl solution at 4 C. The extracts thus obtained were analyzed and fractionated by column chromatography using "DEAE" cellulose and by agar gel electrophoresis. Over two-thirds of the extractable material (54 percent of the delipidated powder) was found to be water-soluble. The major soluble constituents were identified as glycoproteins.

The antigenicity of the total extracts as well as of the fractions obtained by chromatography and by electrophoresis, was tested by agar-gel double diffusion and immunoelectrophoretic analyses, against sera from experimentally infected animals and from natural human infections. The specificity of these reactions is discussed.

68. **Isolation of Specific Antigens from *Ascaris lumbricoides*.** H. N. KENT, The Johns Hopkins University, School of Hygiene and Public Health.

Among the natural substances found in living organisms, proteins and protein complexes are known to possess high antigenic potential. If the concept of immunological specificity is based on the intricate structure of the protein antigens as well as their nature, then it is conceivable that a specific interaction between the antigen and the corresponding antibody would only be possible when the antigen retains its native configuration. Based on these considerations and the previous experiences in this laboratory, an attempt was made to extract and isolate somatic native proteins from *Ascaris lumbricoides* and to evaluate their antigenicity by means of physico-chemical and immunochemical techniques.

Fresh *Ascaris lumbricoides* var. *suum*, females, obtained from the local slaughter houses were extracted with triple distilled water at 4 C. after previous homogenization and delipidation with absolute ethanol and ether at −70 C as described elsewhere. The water extract analyzed by paper and agar-gel electrophoresis showed six distinct groups of protein which were separated and isolated by column chromatography with "DEAE" cellulose, and by preparative agar-gel electrophoresis. The chemical composition of these fractions as well as their antigenic potential was studied. The specificity of the reactions observed in Ouchterlony's double diffusion tests, in immunoelectrophoresis, and in intradermal reactions on humans with natural ascaris infections is considered.

69. **Immunity in White Mice Produced by Feeding Irradiated *Ascaris* Larvae.** J. B. VILLELLA, H. J. GOMBERG, AND S. E. GOULD, Phoenix Memorial Laboratory, University of Michigan.

In the present study, an immunity to *Ascaris* infection was induced in mice by initial infection with second stage *Ascaris* larvae irradiated with gamma rays from cobalt 60.

Six groups of 8 to 10 mice each, 5 to 6 weeks of age, were given approximately 10,000 infective larvae of *Ascaris suum* irradiated with 80,000, 100,000, 120,000, 160,000 180,000 and 220,000 rep (roentgen equivalent physical) of gamma radiation, respectively a control group was infected with an equal number of non-irradiated larvae. On the seventh day after infection, half of each group of mice were killed and the number of larvae developing in the liver and lungs was determined. The average numbers of larvae recovered from both the liver and lungs in the groups of test mice were 888, 868, 1054, 40, 194 and 105, while the average number of larvae recovered from the control group was 3,224. The remaining half of each group of mice were then challenged on the 14th day after the first infection by feeding each of them 10,000 non-irradiated larvae. The average numbers of larvae recovered from the test groups on the seventh day after challenge was 157, 307, 157, 182, 148 and 122, respectively; while the average number of larvae in the control group given the challenge infection was 127. A second control group of mice, not previously infected, was fed with the same number of non-irradiated larvae from the same batch as the challenge dose; the average number of larvae recovered from the liver and lungs 7 days after this infection was 2,910.

The lungs of control animals fed non-irradiated larvae disclosed numerous petechiae and from 75 percent to total consolidation following the initial infection, while the lungs of mice which were fed larvae irradiated with 180,000 or 220,000 rep showed a few or no petechiae and no consolidation. Few new petechiae were found in the previously infected groups of test and control animals given the challenging infection. (Supported by Michigan Phoenix Project, University of Michigan, and Pathology Research Fund, Wayne County General Hospital.)

70. **Attempts at Axenic Culture of Lungworms of Swine.** H. G. SEN AND G. W. KELLEY, JR., University of Nebraska.

Third-stage larvae of *Metastrongylus apri* and *pudendotectus* were removed from the earthworm tissue by gastric digestion and aseptically inoculated into axenic systems. Chick embryo extract (Weinstein, 1959, J. Parasit.), Hanks medium (Hanks et al, 1955, An Introduction to Cell and Tissue Culture, Burgess), trypticase medium (Diamond and Douvres, U.S.D.A.), and a combination of Hanks and trypticase media were tested.

Almost no molting took place in the chick embryo extract medium, but most of the original third-stage larvae survived the 8-day test in this medium.

Hanks medium promoted the best growth. Fifteen percent of the larvae had reached fifth stage in this medium by 192 hours. Survival was low in Hanks; nearly all of the larvae were dead following the 8-day test.

Trypticase-Hanks medium gave nearly as good growth as Hanks alone. Sixteen and 10 percent of the larvae had molted into fourth- and fifth-stage larvae respectively by the eighth day. Almost all of the larvae were alive at this time and some grew to maturity when inoculated directly into the bronchus of a pig.

Molting did not take place in the trypticase medium alone but almost all were alive in this medium after 8 days.

71. **In Vitro Culture of the Larvae of *Ascaris lumbricoides suum*. A Report on Progress.** T. D. PITTS, Loyola University of Los Angeles.

Axenic cultivation of newly hatched *Ascaris* larvae in complex organic (oligidic) media produced increases up to 30 percent in length and 25 percent in diameter (Pitts and Ball, 1955). More recent tests with similar media yielded increases up to 50 percent in length and 150 percent in diameter. Some cultivated larvae attained lengths produced by some larvae in the livers of mice by the fifth day of an infection.

Larvae, hatched in the intestines of mice and axenized, did not grow or survive as well as many artificially hatched larvae.

Presently, a comparison is being made of the growth in axenic cultures of newly hatched larvae and of 4-, 6- and 8-day old larvae from the livers and lungs of infected mice. The larvae have been cultured in roller tubes in media consisting of hog serum and CEE, alone, or supplemented with a variety of organic materials. Subcultures have been made daily or every other day.

Poor survival and growth have resulted with newly hatched larvae, but many older larvae have shown increases in length of 100 percent to 200 percent. The largest size (2407 microns

by 70 microns) was obtained in an 18-day culture of larvae from the liver of an 8-day mouse infection. This represented a 282 percent increase in length and a 93 percent increase in diameter.

A few free sheaths have been found in some cultures, but no active exsheathing has been observed. (Supported in part by a research grant, E1037, from the National Institute of Allergy and Infectious Diseases, Public Health Service.)

72. **Cultivation of Parastic Stages of the Swine Nematodes** *Hyostrongylus rubidus* **and** *Oesophagostomum quadrispinulatum* (*O. longicaudum*) **Free of Microbial Associates.** L. S. DIAMOND AND F. W. DOUVRES, Animal Disease and Parasite Research Division, A.R.S., U.S.D.A., Beltsville, Maryland.

Eight-to 9-day-old infective third-stage larvae of *Hyostrongylus rubidus* and *Oesophagostomum quadrispinulatum* were isolated from sphagnum-moss cultures seeded with feces of swine infected with both species. After the larvae were exsheathed by hypochlorite treatment, they were introduced into a medium that contained principally, trypticase, yeast extract, glucose, sheep serum, and extracts of rabbit embryo and pig liver. Bacterial and fungal associates that survived hypochlorite treatment were eliminated by the use of penicillin, streptomycin, and mycostatin.

Within 42 days at an incubation temperature of 38.5 C, *H. rubidus* reached the fourth molt and *O. quadrispinulatum* the fourth stage. This time interval was considerably longer than the one required for comparable in vivo development as reported by others. Additional differences observed included stunted development from the fourth stage onward of larvae grown in vitro and failure of the genital primordium of *H. rubidus* grown in vitro to keep pace with somatic differentiation. Lack of information on the in vivo development of the *O. quadrispinulatum* genital primordium precluded a similar comparison.

73. **The in vitro Cultivation of Some Gastrointestinal Nematodes of Cattle and Sheep.** F. W. DOUVRES, Animal Disease and Parasite Research Division, A.R.S., U.S.D.A., Beltsville, Maryland.

Utilizing most of the procedures for in vitro cultivation of nematodes and the medium described by Diamond and Douvres (Abstract No. 1, this issue), modified as stated below, development of certain immature parasitic stages of several nematodes of cattle and sheep has been induced artificially. Serum from cattle supplanted sheep serum in the medium and a vitamin mixture (Weinstein and Jones, 1959, N. Y. Acad. Sci. 77: 137) was added for some tests. Infective larvae of *Oesophagostomum radiatum* and of two ovine strains of *Haemonchus contortus*, one resistant (R) and the other non-resistant (NR) to phenothiazine (Drudge and Leland, 1958, J. Parasit. 44: 40), were cultured separately. Also cultured was a mixture of larvae containing *Ostertagia ostertagi*, bovine *H. contortus* and two unidentified bovine *Cooperia* spp. Larvae of bovine origin were cultured in both vitamin-supplemented (SM) and non-supplemented (NSM) medium, those of ovine origin in supplemented medium only.

The most advanced developmental stage reached by each species or strain in either or both types of medium and the earliest day after incubation (DAI) that this stage was attained was: Bovine *H. contortus* and *O. ostertagi*, fourth stage, 9 DAI in NSM and 7 DAI in SM; *Oes. radiatum*, third molt, 14 DAI in NSM and "late" fourth stage; 27 DAI in SM; *Cooperia* spp. fourth stage, 9 DAI in NSM; ovine *H. contortus*, fourth molt, 27 and 7 DAI for R and NR strains, respectively.

Comparisons with available reported data on in vivo development showed that in culture: Time required for attainment of the advanced stage was longer, development of the genital primordium lagged behind somatic differentiation, and larvae in fourth stage and fourth molt were stunted.

74. **Influence of intestinal extracts and sera from cattle infected with** *Oesophagostomum radiatum* **on the in vitro cultivation of this nematode: Preliminary Report.** F. W. DOUVRES, Animal Disease and Parasite Research Division, A.R.S., U.S.D.A., Beltsville, Maryland.

Extracts were added to a vitamin-supplemented culture medium (SM) previously used for cultivation of this and other nematodes (Abstract No. 2, this issue); test sera replaced the normal serum ingredient of this medium. Controls used included extracts and sera from helminth-free cattle and the extractive agents, tested both for intrinsic and dilution effect.

Tested were: (1) Phosphate buffer extract (PBE) of pooled mucosal and submucosal scrapings from the combined small intestine, cecum and colon of a young helminth-free calf (C1); (2) PBE, saline extracts (SE) and acetone powder buffer extracts (APBE) of homogenates of similar scrapings from the small intestine, and PBE and SE of homogenates of similar scrapings from the cecum and colon of three long yearlings, one (C2) reared helminth-free,

one (C3) infected with *O. radiatum* larvae once and harboring a patent infection, and one (C4) rendered resistant to reinfection with this nematode by repeated doses of its larvae; (3) distilled water extract (DWE) of mucus and partially digested food particles washed from the mucosal surface of the intestinal tract of each yearling; and (4) inactivated sera from blood collected from each yearling immediately before slaughter. Tissue homogenates from C3 and C4 included the nodules produced by the host against this parasite.

In the medium containing PBE of C1 and in the buffer control, *O. radiatum* reached the late parasitic third stage, third molt, early fourth stage, fourth molt and fifth stage, 4, 6, 13, 22, and 24 days after incubation (DAI). Nearly identical rates of development to the first three aforementioned stages occurred in the media serum C2 and extractive-agent controls. Growth and rate of development to these stages was accelerated markedly by DWE from C2 and C3 and somewhat less by that from C4. However, a definite lag in the development to these stages occurred in media containing sera and cecum-colon PBE from C3 and C4.

As early as 3, and persisting at 19, DAI, in all tests involving tissue extracts of C3 and C4, most larvae in the third stage and third molt had ribbon- and plume-like aggregates of refractive precipitates at their body openings or adhering to the body surface. Additionally, most larvae of all stages in these cultures were never free of "media debris" which coated the body surface. Both the precipitates and "coating" were always present; these were formed most profusely within 24 hours after each transfer and tended to diminish with time between transfers. Such reactions were not observed on larvae developing in media containing DWE or tissue extracts from C2, DWE or sera from C2, C3, or C4 or in any test controls. These reactions are in part analogous to those observed by Sarles (1938, J. Inf. Dis. **62**: 337) for *Nippostrongylus muris* in immune rat serum.

75. **Preliminary Studies on the Ecology of *Leidynema appendiculata* (Leidy, 1850) (Nematoda: Oxyuroidea).** C. M. FAY AND F. J. ETGES, University of Cincinnati.

The present study reveals that *Leidynema*-free *Blatta orientalis* can not be cultured from isolated oothecae of this roach obtained from the Army Research and Engineering Laboratory, Natick, Mass. Careful precautions were taken against external contamination of the oothecae, including rinses in detergent and dilute alcohol. It is, therefore, suggested that *Leidynema* eggs may be transferred within the egg cases of their host. Dissection of roach oothecae did not reveal any nematode eggs. In their description of the life history of *Leidynema appendiculata*, Dobrovolny and Ackert (1934, Parasit. **26**: 468–480) found no evidence of this transfer mechanism.

It was further noted in the present study that *Leidynema* populations were decreased by experimentally infecting roaches with the protozoa *Nyctotherus ovalis* and *Lophomonas* spp. Protozoan-infected roaches had an average of 5.7 worms, while protozoan-free controls had an average population of 18.2 *Leidynema* adults.

Attempts to obtain *Leidynema*-free roaches by means of anthelmintics administered in drinking water were unsuccessful. Piperazine tartrate (25 mg per ml) and piperazine citrate (10 mg per ml) had no effect on worm populations. Roaches given crushed fresh garlic in water showed no decrease in the number of *Leidynema*, but autoamputation of the host antennae was noted.

In a brief study of feeding habits in adult *Leidynema*, worms were exposed in vitro to suspensions of Congo Red-stained yeast, India ink, and protozoa from the colon of *Blatta orientalis*. No indication of ingestion of particulate food was found.

76. **A Survey of Parasitism in Skunks (*Mephitis mephitis*) in Louisiana, With Observations on Pathological Damages Due to Helminthiasis.** B. B. BABERO, Southern University, Baton Rouge, Louisiana.

Active investigations are in progress relative to the occurrence, distribution, and prevalence of parasites in Louisiana skunks To date, over 126 mature animals have been examined from localities throughout the state. Tentative identifications of the endoparasites collected and the approximate number of animals infected are as follows: NEMATODA, *Arthrocephalus lotoris* (27), *Ascaris columnaris* (56), *Dipetalonema* sp. (6), *Filaroides martis* (1), *Gnathostoma* sp. (3), *Gongylonema longispiculum* (27), *Molineus barbatus* (45), *M. patens* (69), *Physaloptera maxillaris* (118), *Strongyloides* sp. (12), and *Trichinella spiralis* (1); TREMATODA, *Alaria taxideae* (22), *Brachylaime virginianum* (2), and *Psilostomum* sp. (30); CESTODA, *Mesocestoides variabilis* (82), *Oochoristica oklahomensis* (14), and unidentified cestode larvae (liver, 1); ACANTHOCEPHALA, *Macracanthorhynchus ingens* (4), *Pachysentis canicola* (25), and unidentified Acanthocephala (2); PROTOZOA, *Trypanosoma cruzia* (10).

The occurrence in the skunk of *B. virginianum, G. longispiculum, M. barbatus,* and *Psilosto-*

mum sp. apparently represent new parasite records for this host. In addition, *Psilostomum* sp., and perhaps *Gnathostoma* sp., may be new to parasitological literature.

Filaroides martis, Gnathostoma sp., *Molineus patens,* and *P. maxillaris* were observed to be highly pathogenic to skunks. *Molineus patens* ulcerates and/or perforates the intestinal wall and frequently induces fibrous tumor formation both on the mucosal and serosal surfaces of this organ. To a lesser extent, most other parasites of skunks were observed to cause some pathological damage. The bionomic conditions existing in Louisiana, coupled with the omnivorousness of the skunk favor massive helminthic infections, which at the same time threatens the population of these animals. Skunks, in acting as a reservoir of infection, possibly intect livestock and other mammalian species of the state.

77. **Effects of pH, Various Proteolytic Enzymes, Amino Acids and Other Substances on the Eggs and Larvae of** *Ascaridia galli.* J. C. HWANG, Animal Disease and Parasite Research Division, A.R.S., U.S.D.A., Beltsville, Maryland.

Experiments were conducted to determine the ability of *Ascaridia galli* eggs to hatch in vitro in media consisting basically of physiologic saline, dextrose, and sodium bicarbonate, to which was added (1) one of the following proteolytic enzymes or other substances—pepsin, trypsin, sodium taurocholate, duodenal powder or ox bile; or (2) one of the following amino acids—serine, proline, tryptophane, leucine, or tyrosine. After the media were adjusted to a pH of 7.2, 100 embryonated eggs were added to each medium and incubated at 40 to 42 C. Examinations for hatched larvae were made from 0.5- to 6-hour intervals. Except in those dishes containing either duodenal powder or tyrosine, in which no larvae were found, the rest of the media showed active larvae. With the exception of trypsin, the number of larvae hatched in most of the others was extremely low. Out of seven trials with the solution containing trypsin and with pH adjusted from 6.5 to 7.5, only the four with a pH range of 6.8 to 7.2 produced positive results. Hatching of larvae in these solutions occurred at the end of the third hour of incubation.

The pH of contents of digestive tracts from freshly killed, nonparasitized, 4-week-old chicks was 2.89 to 4.14 in the proventriculus, 2.72 to 4.00 in the gizzard, 6.08 to 7.15 in the duodenum, 6.10 to 7.28 in the jejunum, and 5.88 to 7.42 in the ileum. Preliminary examinations of four 4-week-old chicks, killed from 45 minutes to 5 hours after inoculation with undetermined numbers of embryonated *A. galli* eggs, revealed that more than 75 percent of hatched larvae occurred in the lower portion of the jejunum, where the pH ranged from 6.7 to 6.9.

However, additional observations on ten other 4-week-old chicks killed 12 to 16 days after inoculation with a single dose of 1,000 embryonated eggs each disclosed that 6 percent of the overall average number of worms were found in the duodenum, 53 percent in the jejunum, and 41 percent in the ileum; none were found in the proventriculus or gizzard. The average pH of the contents was 2.97 in the proventriculus, 2.67 in the gizzard, 6.27 in the duodenum, 6.47 in the jejunum, and 7.11 in the ileum. The smaller percentage of worms found in the jejunum as compared to the number found earlier might have been caused largely by their migration to other parts of the intestine. These observations indicate that the pH factor may prove to be of considerable importance in stimulating the hatching process and in the distribution of young larvae in the intestine of their host.

78. **Amino Acids in** *Ascaris suum* **eggs.** B. J. JASKOSKI, Loyola University, Chicago.

Chemical separation of the inner, middle and outer membranes of the eggs of *Ascaris suum* was followed by hydrolysis in acid and alkali. Larvae from embryonated eggs were also hydrolyzed. Ascending and two-dimensional paper chromatography revealed the presence of 18 natural amino acids in the outer membranes and in the larvae. Nine amino acids were identified from the inner egg membrane, previously supposed to be lipoid in nature. Further analysis of the inner fraction using the dinitro-fluoro-benzene technic revealed the arrangement of the amino acids in the inner membrane. (Research supported by USPHS, National Institute of Allergy and Infectious Disease, Grant E-1826.)

79. **Susceptibility and Resistance of** *Anopheles quadrimaculatus* **to** *Dirofilaria uniformis.* R. E. DUXBURY, A. P. MOON AND E. H. SADUN, Walter Reed Army Institute of Research, Washington, D.C.

Some of the factors which influence the development of *Dirofilaria uniformis* in the invertebrate host were studied.

A microfilaremia of between 100 and 150 per 0.02 ml of blood was found to be the most suitable to produce infective larvae in *Anopheles quadrimaculatus* in sufficient numbers to insure routine transmission of the infection. When 120 mosquitoes were fed on a rabbit with over

3,000 microfilariae per 0.02 ml of blood, 92 died within 3 hours, and only five survived to the tenth day.

After 10 to 12 days, 12,765 infective larvae dissected from 508 mosquitoes were distributed as follows: head, 54 percent; thorax, 20 percent; and abdomen, 26 percent. Larval filariae collected at 10 days either from the head, or from the thorax and abdomen were equally infective for rabbits when injected subcutaneously.

When infected mosquitoes were fed on normal rabbits, there was a considerable loss of larvae from the thorax and abdomen as well as from the head.

The number of larvae recovered from mosquitoes maintained at 27 C was considerably higher than in the groups kept at 24 C and 31 C. Larvae developing at 31 C grew more rapidly during the first 7 days than those at the lower temperatures, but by the tenth day the larvae at 27 C had attained the greatest mean length.

Fewer new larvae developed in super infected mosquitoes than in the previously uninfected controls. These differences, however, are not statistically significant.

An inverse relationship was observed between the age of mosquitoes at infection and the number and length of the larvae developing 3, 6, and 9 days later.

80. **The Incidence of *Spirocerca lupi* and *Dirofilaria immitis* in Dogs in the Philippine Islands.** W. S. Bailey, Auburn University.

Dogs from the city pound in Manila, Philippine Islands, were examined during the period February 15 to May 5, 1959, for infection with *Spirocerca lupi* and *Dirofilaria immitis*. Almost all of these were native dogs; all had been collected from the streets from all areas of the city. No attempt was made to estimate the age of each dog except to eliminate from the study all dogs less than 6 months old. *Spirocerca* infection was demonstrated in 147 of 322 dogs (45.5 percent). Adult *Dirofilaria* were found in the hearts of 40 of 210 dogs examined (19 percent).

Examination of necropsy protocols for the years 1921 to 1934 and 1948 to 1958 at the College of Veterinary Medicine, University of the Philippines, revealed that *Dirofilaria* had been recorded for 69 of 348 dogs which were at least 6 moonths old (19.8 percent). The records were incomplete with reference to information on *Spirocerca* infection and no comparison could be made of the infection rate in the medical cases and that of the pound dogs.

Information will be presented on the reported geographic distribution and incidence of *Spirocerca* infection in other countries.

81. **Canine Microfilariasis in Eastern United States.** N. Rothstein, K. E. Kinnamon, M. L. Brown and R. W. Carithers, Walter Reed Army Institute of Research, Washington, D.C.

Using the acridine orange vital staining technique described by Rothstein (1958. J. Parasit. **44**: 588–595) for the detection and differentiation of microfilariae (Rothstein et al, 1959, J. Parasit. **45** (supp.): 38), this report presents the results obtained with the vital staining technique of a 3-year survey of the incidence of canine microfilariasis in Eastern United States.

A total of 1318 dogs were surveyed, 600 from Alabama, 649 from Pennsylvania and 69 from Rhode Island. Of those from Alabama, 244 (40.7 percent) harboured microfilariae. Of 555 differentiated samples, 6 (1.1 percent) were *Dirofilaria immitis* and 210 (37.8 percent) were *Dipetalonema* n. sp. Only 31 (4.8 percent) were positive in the Pennsylvania group. Eleven (1.7 percent) were *D. immitis* and 17 (2.6 percent) were *Dipetalonema* sp. A third type, shorter and wider than *Dipetalonema*, but which moved and stained with Coriphosphine like *D. immitis*, was found in three (0.5 percent) dogs. Two (2.9 percent) of the 69 from Rhode Island harboured microfilariae. One (1.5 percent) was *Dipetalonema* and the other had a mixed infection of *D. immitis* and the third type mentioned above.

No statistically significant difference was found in the incidence of diromicrofilariasis (heartworm) in the North or South, within the areas surveyed. The incidence of dipetalomicrofilariasis in Alabama was about 15 times higher than in the North. The evaluation of canine microfilariasis in the United States must also consider the existence of yet a third type of microfilariae.

82. **Maintenance of *Dirofilaria uniformis* in Laboratory Animals.** A. P. Moon, R. E. Duxbury, and E. H. Sadun, Walter Reed Army Institute of Research, Washington, D. C.

Investigations were conducted to find a suitable laboratory animal in which infection with *D. uniformis* could be maintained routinely. After studying the development of infection in its natural host, the cottontail rabbit (Bray and Walton, in press), unsuccessful attempts were made to infect young mice, rats, hamsters and guinea pigs. A few larvae survived in suckling mice up to 7 days following inoculation without visible development.

Twenty young adult and suckling domestic laboratory rabbits were given between 50 and 1,031 infective larvae each subcutaneously and sacrificed at intervals up to 322 days. The larvae developed into mature adults in the subcutaneous tissue and produced microfilariae which appeared in the peripheral blood between 116 and 150 days after inoculation. However, the number of microfilariae was always too low to produce infective larvae in mosquitoes in sufficient numbers to insure a routine transmission of the infection. Most of the worms in these rabbits became encapsulated and disintegrated within the period of observation. Attemps to interfere with the host response by injections with cortisone were unsuccessful.

Very promising results were obtained when suckling Dutch breed domestic rabbits were used. Within 4 months from the date of infection, microfilariae were present in the blood in sufficient numbers (more than 50 per 0.02 ml) to infect *Anopheles quadrimaculatus* mosquitoes adequately. The relatively high microfilaremia was maintained for several months and many groups of mosquitoes were infected. The above results indicate that Dutch rabbits can be used as suitable laboratory hosts for experimental studies with *D. uniformis.*

83. **The Effect of Three Different Exposures to Parasitism on the Serum Proteins of Shropshire Lambs.** J. H. Turner and G. I. Wilson, Animal Disease and Parasite Research Division, A.R.S., U.S.D.A., Beltsville, Maryland.

A 10-month study was conducted to determine the typical serum protein values for lambs raised under three types of management which resulted in exposure to various degrees of parasitism. Lambs of the first, or isolation group, were taken from their ewes at birth and raised in a helminth-free isolation building. These lambs acquired only light coccidial infections. Lambs of the second, or barn group, were maintained in concrete-floored pens and acquired minimal infections of *Strongyloides papillosus* and various coccidia. Lambs of the third, or pasture group, were raised with their ewes on pasture and were exposed to relatively heavy parasitic infection with various trichostrongyles, *Oesophagostomum* spp., and *S. papillosus.* All groups after weaning were fed mixed grain; the groups in the isolation house and barn receiving alfalfa hay instead of green forage.

Average gamma globulin percentages were relatively constant in lambs of the isolation and barn groups throughout the period studied; the isolation group averaging consistently lower. Terminal readings were 13 percent and 16 percent for the isolation and barn groups, respectively. In the pasture group, the gamma globulin percentage rose steadily after the fourth month and had reached a maximum of 46 percent when the study was completed.

Average albumin to globulin ratios (A/G) declined steadily in lambs of the heavily parasitized pasture group but increased steadily in the other two groups. Average terminal A/G ratios were 3.2, 2.4, and 0.4 for the isolation, barn, and pasture groups, respectively.

These results show that the gamma globulin percentages and the A/G ratios, were influenced by the three management systems studied, the greatest differences occurring in those lambs exposed to the relatively heavy parasitic infections.

84. **Diagnosis of Experimental Stephanuriasis in Swine by a Double Diffusion Agar Precipitin Technique.** F. G. Tromba and L. A. Baisden, Animal Disease and Parasite Research Division, A.R.S., U.S.D.A., Beltsville, Maryland.

Ante mortem diagnosis of *Stephanurus dentatus* infection in swine has hitherto been difficult and sometimes impossible because often the prepatent period, which is usually in excess of 6 months, is longer than the life of a market animal. Tromba [1958, J. Parasit. **44** (supp.): 29] reported that an eosinophilia, which reached peak values about 4 weeks after infection, could be demonstrated in swine experimentally infected with *S. dentatus.* However, this cannot be considered pathognomonic since other helminths, particularly *Trichinella,* also elicit an eosinophilia in swine.

A diagnostic technique utilizing the micro-Ouchterlony methods of Wadsworth (1957, Internatl. Arch. Allergy and Appl. Immun. **10:** 355–360) and Crowle (1958, J. Lab. Clin. Med. **52:** 784–787) has been developed, which within limitations will give a specific reaction. Sera obtained from swine 4 to 14 weeks after experimental infection with kidney worms contained antibodies, which formed precipitates with antigens prepared from juvenile and adult kidney worms. The position and shape of the specific precipitin lines are constant throughout this period, although some variation in number has been observed between sera.

Sera of swine experimentally infected with *Ascaris suum, Trichuris suis, Hyostrongylus rubidus, Trichinella spiralis, Oesophagostumum quadrispinulatum, Metastrongylus apri,* and *M. pudendotectus* have been tested against these kidney worm antigens. In all cases, precipitin lines have been found in these heterologous systems. However, differences in their position and shape make them readily distinguishable from lines formed in the homologous system.

85. **The Pathology Occurring in Lambs as a Result of Inoculation with Embryonated Eggs of *Ascaris lumbricoides* var. *suum*.** P. R. FITZGERALD, Animal Disease and Parasite Research Division, A.R.S., U.S.D.A., and the University of Illinois.

Two experiments were conducted to observe the symptoms and gross and histologic pathology associated with the migration of larval ascarids in lambs following inoculation with large doses of embryonated eggs. Larval migration and the development of the immature stages were observed as has been reported from other experimental animals. No adult worms were recovered. Dyspnea and reduced food consumption were observed between the fourth and eighth days post inoculation. In one lamb, a maximum temperature of 106.9 F was observed on the eighth day post inoculation; another died 8 days post inoculation when third-stage larvae were most abundant in the lungs.

No consistent changes were observed in hemoglobin, packed cell volume, or total white cells. The eosinophil-neutrophil ratio began to show a change in blood samples collected on the eighth day after inoculation. In one animal, the eosinophil count reached 23 percent, and there was a concurrent decline of neutrophils to 15 percent.

At necropsy, gross pathological changes were observed chiefly in the liver and lungs. Swelling and severe petechial and ecchymotic hemorrhages occurred in the lungs in animals necropsied on the fourth and eighth days. In animals necropsied 16 days or more post inoculation, there was no evidence of gross pathology.

Microscopic examination of lung tissues revealed congestion and hemorrhage, infiltration with lymphoid and plasma cells, and some desquamation of the bronchiolar epithelium. The liver most frequently showed congestion, fatty metamorphosis with infiltration by polymorphonuclear leukocytes. Changes in other tissues were minor or not detectable.

86. **The Detection of Krebs Cycle Intermediates in the Incubation Media of *Haemonchus placei* Larvae after Incubation with C^{14}-Labeled Substrates.** W. M. DICKSON, J. S. DUNLAP, AND T. GORDON, Washington State University.

Third-stage larvae of *H. placei* were incubated with radioactive glucose (uniformly labeled-C^{14}) or pyruvic acid-3-C^{14} in a Warburg respirometer. After 3 hours of incubation, radioactivity was measured (1) from the center-well fluid to ascertain $C^{14}O_2$ production (2) in the incubation media to ascertain substrate uptake and metabolite excretion (3) in the worms to measure absorption. The supernatant fluid (incubation media) was subjected to celite column chromatography using gradient elution with chloroform-butanol to separate the various Krebs Cycle intermediates. The various peaks were determined by titration and the radioactivity in each peak was determined by liquid scintillation methods.

Using labeled glucose as the substrate, acid fractions were recovered from the column having peak effluent volumes resembling acetate, lactate, pyruvate, citrate, α-ketoglutarate, succinate, fumarate, and l-malate. Activity was recorded in the pyruvate and citrate fractions. When pyruvate-3-C^{14} was utilized as the substrate, fractions with peak effluent volumes resembling the above acids were likewise recovered but radioactivity was measured in the pyruvate, citrate, α-ketoglutarate, succinate, fumarate, and l-malate fractions.

Limited recovery trials resulted in a 35 percent recovery as measured by C^{14} activity. Radioactivity was consistently recovered from the center well fluid and from the worm; in the latter case, chromatographic separations have not been completed.

87. **Effect of Cortisone on the Susceptibility of Hamsters and Guinea Pigs to the Sheep and Rabbit Strains of *Strongyloides papillosus*.** B. BEZUEIK, Rockefeller Foundation Fellow, Animal Disease and Parasite Research Division, A.R.S., U.S.D.A.

The writer investigated the susceptibility of golden hamsters and guinea pigs to infection with the intestinal threadworm of ruminants, *Strongyloides papillosus* (Wedl, 1856). The objective was to determine if these small abnormal hosts could be substituted for the larger laboratory rabbit as test animals. Attempts to infect percutaneously ten 2-month-old hamsters and four 1-month-old guinea pigs by exposing them to 2,000 to 50,000 infective larvae of the sheep strain were unsuccessful. Only two became infected. Eggs were not found in the feces, nor was the parasite recovered from animals necropsied 9, 15, 22, and 30 days after infection. Since other workers, e.g., (Coker, 1955, J. Parasit. **41**: 498–504) had reported that cortisone decreased the host's natural resistance to helminth infection, ten 3-month-old hamsters and five 1-month-old guinea pigs were injected daily with 0.5 mg and 1.0 mg hydrocortisone sodium succinate. The hormone was given intramuscularly, beginning 2 days before percutaneous infection with 5,000 to 50,000 infective larvae of *S. papillosus*, and administered until the animals were sacrificed. Two guinea pigs, similarly treated with cortisone, were exposed to 10,000 to 25,000 infective larvae of the sheep strain of *S. papillosus* after one passage through the rabbit. Eggs of *S. papillosus*

were not found in the feces, nor was the parasite recovered from animals necropsied 1, 2, 4, and 6 weeks after infection. The author believes this report is the first to show that the natural resistance of hamsters and guinea pigs to *S. papillosus* is apparently not reduced by the intramuscular injection of cortisone at the dosage indicated.

88. **Preliminary Studies with Snails on Chemotaxis and Chemotactic Agents.** F. J. ETGES, University of Cincinnati.

Based on the suggestion of W. A. Mozely (1953, H. K. Lewis, Pub. London), that control of snails may be possible through the use of specific attractants, the present study was undertaken to determine the existence of attractive substances and their identity. Cultures of the schistosome vectors, *Taphius* (*Australorbis*) *glabratus* and *Bulinus* sp., as well as local pond snails (*Physa* and *Helisoma*) are used.

Snails in polyethylene-lined tanks are experimentally subjected to various crude and fractionated materials placed at distances of up to 30 feet. The rate at which snails move toward the "bait" and the percentage of snails which reach it are used as an index of attraction, when compared with simultaneous control experiments using no attractant.

At present, evidence is inconclusive with respect to the ultimate utility of this data, as is the specific chemical identity of the attractant principle. (This study is supported by a Research Grant from the National Science Foundation.)

89. **Biochromes in Certain Species of Larval Trematodes.** A. M. NADAKAL, University of Southern California.

An attempt has been made to study the chemical nature and sources of pigments in 10 species of larval trematodes found in the marine snail, *Cerithidea californica*. The sources of carotenoid and chlorophyllic pigments have been studied by way of the food-chain cycle involving the snail host and four species of algae. After extraction with suitable solvents and chromatographic separation, pigments were identified by spectrophotometric absorption analyses and other methods. β-carotene was extracted from eight species of larval trematodes. Only three species of rediae and none of the sporocysts contained chlorophyll derivatives. A keto-caroteniod was also identified from one of the sporocyst types. All available evidence indicated that the parasitic larvae absorb pigments from the snail host. They appear to be selective in absorbing only the hydrocarbons from several kinds of carotenoids available in the snail. No significant metabolic alteration of pigments in the larvae has been noticed. Various histochemical tests have shown that the brownish pigments located in the eyespots and body tissues of three species of cercariae are melanins. Tyrosine, a precursor of melanin, has been found in all the nine species of cercariae studied. However, the results obtained for the presence of tyrosinase were inconclusive. The fate of eyespots in *Euhaplorchis* cercaria has been followed through the metacercarial stage, recovered from the intermediate host, *Fundulus*, over a period of 6 weeks. Tests for flavones, riboflavins, haemoglobin, bile pigments, lipofuscines, and haemocyanins were negative in all the larval species. (This abstract is made from a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Southern California, 1959).

90. **The In Vitro Action of Normal and Immune Vertebrate Sera on Cercariae Naturally Infecting** *Cerithidea californica*. W. B. LEFLORE, University of Southern California.

Cercariae from the mud flat snail, *Cerithidea californica*, were studied in vitro in normal and immune vertebrate sera. The reaction of *Parorchis avitus*, heterophyids, schistosomes, strigeids, and xiphidiocercariae was studied by ordinary and phase microscopy in fresh and stored normal sera of the toad, *Bufo boreas*, chicken, pigeon, cat, rabbit, and man. All sera except that of the cat exhibited some degree of cercaricidal effect against one or more types of cercariae within one hour. Types of reactions included inactivation of cercariae, spastic movements, dropping of tails, cytoplasmic granulation, distortion of shape, oral precipitate production, agglutination, and death. Abortive cysts were produced when the cercariae of *Parorchis avitus* were immersed in cercaricidal sera. Serum inactivation at 56 C rendered it completely ineffective in most cases; however, in others the inactivation did not totally remove the cercaricidal effect. This suggested that some factor associated with the heat stable components of complement, possibly properdin, contributes to these reactions.

The action of *Schistosoma mansoni* human immune serum was studied against the schistosomes from *C. californica*. The cercariae produced oral sticky excretions which caused them to adhere to one another, probably resulting from a weak immune reaction. Antisera produced in chickens by injection of a cercarial antigen of large pigmented echinostomes immediately produced precipitates around such cercariae. This antiserum when tested against the

cercariae of *Parorchis avitus* and *Cloacitrema michiganensis* produced weak reactions characterized by sticky tail and oral excretions. Heat treatment prolonged the time of formation of the precipitates and sticky excretions. (This research was supported in part by a Fellowship from The National Medical Fellowships, Inc. through funds appropriated by the National Foundation.)

91. **The Effect of a Diamino-Dibenzyl-Alkane on the Histochemical Reaction for Alkaline Phosphatase in Adult** *Schistosoma mansoni.* R. M. LEWERT AND D. G. DUSANIC, The University of Chicago.

It is known that the symmetrical diamino-dibenzyl-alkanes have a high in vitro activity against adult *S. mansoni.* The diamines cause a decrease in glucose uptake in vitro but do not directly inhibit the enzymes of the glycolic cycle of the worm. Studies were made of living worms incubated in horse serum in the presence of diamine and of worm sections treated in a similar manner. These were stained to show relative alkaline phosphatase activity using standard histochemical techniques. Living worms incubated in horse serum alone for three hours showed an intense alkaline phosphatase activity while worms incubated in serum and diamine show only faint or imperceptible alkaline phosphatase activity. If the living worms are transferred from the diamine-horse serum medium after paralysis has occurred but before the death of the worm, these individuals regain normal activity and staining for the enzyme. Sections of the worms in contrast to normal controls when incubated in a diamine-substrate mixture show greatly reduced phosphatase activity. Similar treatment of mouse liver sections show no reduced activity of mammalian alkaline phosphatase. The histochemical observations suggest the possibility that the inhibition of glucose transport is causally associated with the inhibition of the enzyme alkaline phosphatase in the adult worm. (This study was sponsored by the Commission on Parasitic Diseases, Armed Forces Epidemiological Board, and supported in part by the Office of The Surgeon General, Department of the Army, Contract No. DA-49-007-MD-516.)

92. **Ultrastructure in the Tails of Furcocercous Cercariae.** F. J. KRUIDENIER, University of Illinois.

The periodic distribution of a sarcotubular system in the tail muscles of the cercaria of *Schistosoma mansoni* has been described briefly by Kruidenier and Vatter (1960, 4th Internatl. Cong. Elect. Micros.: 332). The investigators noted that a parallel development of the tubules of the sarcoplasmic reticulum was not present in the tail muscles of a plagiorchioid cercaria which they studied. The present report amplifies the studies of *S. mansoni* and extends the previous observations to cercariae of *Schistosomatium douthitti* and to strigeid cercariae.

Naturally shed specimens were fixed in a modification of the veronal buffered osmium of Palade. They were embedded in butyl-methyl methacrylate for sectioning on the Porter-Blum microtome. They were examined with an RCA-EMU 3C electron microscope.

The sarcotubules described in *S. mansoni* are characteristically present in the reticulum of the other furcocercous cercariae. Circular muscles of the tails appear to be penetrated erratically by the sarcotubules. Distribution is sparse. The longitudinal myofibers, in contrast, are penetrated at periodic intervals by the tubular system. The sarcotubules interdigitate with the myofilaments and are closely associated with the cell membranes. There appears to be a correlation of the arrangement of the sarcoplasmic reticular system with the functional activity of the tail muscles of the furcocercous cercariae and with the phylogeny of the Anepitheliocystidia. (This investigation was supported in part by a research grant (E-1319) from the National Institute of Health, Public Health Service.)

93. **Studies on Innate Immunity to** *Schistosoma mansoni.* R. M. LEWERT AND S. MANDLOWITZ, The University of Chicago.

The relative susceptibility to infection with *S. mansoni* is being studied in a variety of hosts in which the physiological state of the acellular elements of the host connective tissue varies or is altered with age, genetic strain, irradiation, hormonal imbalance, or nutritional state. Results to date are largely consistent with the concept that the degree of innate immunity in the mouse is correlated with the state of organization of the connective tissue. Highly significant lesser susceptibility is found in those animals with a dense, highly polymerized connective tissue with relatively small amounts of free water and water soluble components. This is the case in old animals of some strains (e.g. CF-1). The reverse is true of young animals and those old individuals of genetic strains having characteristically "young" connective tissue (e.g. LAF-1). (This study was sponsored by the Commission on Parasitic Diseases, Armed Forces Epidemiological Board, and supported in part by the Office of The Surgeon General, Department of the Army, Contract No. DA-49-007-MD-516.)

94. **Susceptibility of Wild Mammals to Experimental Infection with** *Schistosoma mansoni*. J. I. Bruce, L. M. Llewellyn, and E. H. Sadun, Walter Reed Army Institute of Research and Patuxent Research Refuge.

A total of 67 wild mammals representing 15 species were trapped in Maryland, examined for 2 months for the presence of schistosome eggs in the stools, and subsequently exposed to *S. mansoni* cercariae, together with 200 control mice. None of the trapped animals was found to pass schistosome eggs prior to experimental exposure to cercariae.

The animals tested could be separated into three main susceptibility groups. Those of the first group comprising woodchuck, squirrel, white-footed mouse, meadow vole, and opossum permitted normal development of adults and passed viable eggs with miracidia which were infective for susceptible snails. From 11 to 40 percent of the cercariae to which these animals were exposed were recovered as mature adults. Eggs appeared in the stools as early as 53 days after exposure. Those of the second group comprising house rat, cottontail rabbit, raccoon, chipmunk, jumping mouse, and nutria harbored varying numbers of nearly mature worms but no fertile eggs were found in the stools. From 2 to 19 percent of the cercariae to which these animals were exposed were recovered as adults, most of which were immature. Those of the third group comprising fox and muskrat were very resistant and no adult worms were recovered at necropsy.

The worms developing in the house rat and cottontail rabbit were significantly stunted. Histopathologic studies of the intestine and liver of several groups of animals confirmed the observations made on the relative susceptibility of these hosts.

95. **Culturing Hawaiian Avian Marine Schistosome** *Austrobilharzia variglandis* **Penner in Laboratory Media.** G. W. T. C. Chu, N. Au, and D. E. Watson, University of Hawaii.

Attempts have been made to develop media suitable for in vitro studies on *A. variglandis* from experimentally infected chickens, pigeons, and terns. Two basic media are now on trial. The chicken serum medium (CS), at pH 7.4, consists of 25 percent inactivated chicken serum enriched with 0.1 percent glucose, 65 percent of BBL Mixture 199 with antibiotics and phenol red and 10 percent CEE_{20}. A drop of 2 percent washed chicken RBC was added to each 1 ml aliquot. The bovine serum medium (BS) is the same except that a calf serum previously absorbed with chicken RBC replaces the chicken serum.

With medium change every 2 days, schistosomulae from lungs of a tern developed at 37 C within 3 weeks in the CS medium into juvenile males and females. After 43 days of cultivation, there was no further development toward sexual maturity. Neither did larvae from the skin of infected birds mature nor increase more than 2 to 3 × in size in 2 weeks. Larval development in the BS and other serum media is currently being investigated.

Maintenance of adults was possible in the absence of CEE. In the modified BS medium, the worms appeared more normal than those in the CS medium, and contained hematin deposits in their ceca. In both modified media the adults lived for at least 2 months, but did not lay eggs. A male adult, started in CS medium and then transferred to BS medium without CEE, survived for a record 5.5 months. (This work is supported in part by U. S. Public Health Service research grant #E-2424.)

96. **Some Schistosome Cercariae from** *Gyraulus*. G. Grodhaus, California State Department of Public Health, Bureau of Vector Control.

Snails from two localities in California were examined. Of 2649 *Gyraulus* sp. from Hume Lake (Fresno County), collected during the period 1957, 1958, and 1959, the following infections were recorded: (1) *Cercaria elongata* Brackett, 16 snails; (2) *Gigantobilharzia gyrauli* (Brackett) (=*Cercaria gyrauli*), 4 snails; (3) an unidentified schistosome species, 2 snails. Of 308 *Gyraulus* sp. from Ballard Reservoir (Modoc County), collected in 1959, 2 snails were infected with a similar unidentified schistosome. The unidentified cercariae from both localities have coarse granules in the anterior two pairs of penetration glands and fine granules in the posterior three pairs. They possess seven pairs of flame cells. They resemble several species in the Ocellata group of cercariae, though they differ from any schistosome previously reported from *Gyraulus*.

Cercaria elongata and the unidentified species produced dermatitis when tested on volunteers. There have been discontinuous reports of dermatitis among bathers at Hume Lake since 1950, and there were dermatitis complaints at Ballard Reservoir in 1959. All three species of schistosomes occurred at Hume Lake during the summer of 1957, at which time dermatitis cases also occurred. At Hume Lake *Gyraulus* was the only snail found to be infected with schistosomes during the present study.

97. **Studies on the Standardization of the Intradermal Test for Bilharziasis.** I. G. KAGAN, Communicable Disaese Center, Atlanta, Ga., AND J. PELLEGRINO, Instituto Nacional de Endemias Rurais, Brazil.

Under the auspices of the World Health Organization, a program for the standardization of the intradermal test for bilharziasis has been initiated. A survey was recently carried out in Minas Gerais, Brazil, with infants, children and adults infected with *S. mansoni* by injecting intradermally 0.05 ml of antigen containing 20 to 60 gamma N per ml, measuring the area of the wheal after 15 minutes and recording areas of 1.0 cm^2 or greater as positive reactions. All subjects were tested on the back in the scapular region near the neck. In addition adults were tested on the forearm. The following conclusions were obtained: Antigens prepared in the United States and antigens prepared in Brazil showed the same reactivity in the skin test when the nitrogen contents were adjusted to equal values. Analysis made with antigens prepared by four different methods indicates that all are equal in reactivity when the nitrogen is adjusted. Children under five are not satisfactory subjects for the skin test; girls react less strongly than boys and the response on the back of women is equal to the response on the arm of men. Adult women were simultaneously tested on both the arm and back. These tests showed an average of 1.20 cm^2 on the arm and 1.66 cm^2 on the back. The sensitivity of the test on the arm was 75 percent compared to 92 percent for the back with a 20-gamma antigen. Reasons for accepting the criterion of 1.0 cm^2 to indicate a positive result in the skin test and problems requiring further evaluation such as reducing the non-specific response in the skin of noninfected individuals will be discussed.

98. **Studies on Schistosomiasis.** XVI. Non-reciprocal Acquired Resistance Between *Schistosoma mansoni* **and** *Schistosomatium douthitti*. G. W. HUNTER, III, AND C. J. WEINMANN, University of Florida.

Albino mice (CFW strain) were "immunized" by five exposures to a total of approximately 50 cercariae of *Schistosoma mansoni* or *Schistosomatium douthitti* at 12 to 14 day intervals. About 6 weeks after the last immunizing exposure, mice were challenged with 50 cercariae of either the homologous or heterologous species. Immunizing and challenge controls as well as test animals were sacrificed 40 to 50 days after their final infection and their parasite burdens determined.

Homologous challenge with either parasite yielded equivocal results with respect to reduction in worm burden when compared with controls. When mice were challenged with *S. mansoni* after initial exposures to *S. douthitti*, the numbers of *S. mansoni* recovered were not appreciably different from those in challenge controls. However, when mice were first exposed to *S. mansoni* and then challenged with *S. douthitti*, there was a marked reduction in the number of *S. douthitti* in test animals when compared with the challenge control. Essentially the same pattern was obtained in three similar experiments. (This investigation was supported in part by Grant E-1893 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.)

99. **Schistosomiasis in Louisiana Mammals.** E. A. MALEK, L. R. ASH, H. F. LEE, AND M. D. LITTLE, Tulane University.

Heterobilharzia americana Price, 1929, has been recorded from a lynx in Florida and from raccoons in Texas and North Carolina (Price 1929, 1943; Miller and Harkema, 1960). From the present study it is apparent that this schistosome is common in several southern Louisiana hosts. It has been found in 25 of 36 raccoons and 16 of 22 nutrias collected at Pass a Loutre in the Mississippi River delta. In the same area three dogs were found infected, two by stool examination and one at autopsy.

Deaths attributed to this infection have been observed in one dog and three caged raccoons. In all instances post-mortem findings were similar. Eggs were abundant in the wall of the small and large intestines, and granulomata containing eggs were conspicuous in all parts of the liver. In the dog, eggs were also found in the lungs and kidneys. The occurrence of this schistosome in relatively unrelated animals suggests that additional studies may further extend its host-range.

100. **The Life History of** *Heterobilharzia americana*. H. F. LEE, Tulane University.

In the area where raccoons, nutria, and dogs have been found naturally infected with *Heterobilharzia americana*, the common snails are *Neritina reclivata*, *Physa anatina*, *Tropicorbis* sp. and *Lymnaea* sp. Also present are *Pseudosuccinea columella*, *Ferrissia* sp., *Helisoma trivolvis lentum* and *Gyraulus* sp. Beginning in late April, populations of most of the snails build up during the summer reaching a peak in August. Examinations of 1450 *Neritina reclivata*, 1586 *Tropicorbis* sp., 2309 *Physa anatina*, and 667 *Lymnaea* sp. collected at different times of the year have revealed *H. americana* in only one *Lymnaea* out of 102 collected on 8 May 1960. This

cercaria was found to be identical with *Heterobilharzia americana* cercariae previously obtained from experimentally infected *Lymnaea* and *Pseudosuccinea*. *Physa anatina*, *Tropicorbis* sp., *Australorbis glabratus*, *Neritina reclivata*, and *Helisoma trivolvis lentum* were refractory to infection when exposed to miracidia obtained from the feces and liver of naturally infected hosts. The *Lymnaea* and *Pseudosuccinea* shed cercariae about 3 weeks after exposure.

The eggs, miracidia and cercariae of *H. americana* closely resemble those of *Schistosomatium douthitti*. The cercaria has eye spots located approximately at mid-body; five pairs of penetration glands; six pairs of flame cells, one of which is in the tail stem; furcae with membranous fin-folds along the entire margins (lacking in *S. douthitti*); and minute cuticular spines over the entire body and tail.

101. **The Effect of *Schistosoma haematobium* Infection on Egg-Production in the Snail *Bulinus truncatus*.** H. H. NAJARIAN, The University of Texas, Galveston.

Bulinus truncatus snails, both infected and non-infected with *Schistosoma haematobium* (cercarial shedding), were individually isolated and the egg-production of these snails was followed on a 24-hour basis for over a month in the laboratory. All snails were of a similar size range, were simultaneously collected from the same ditch in the vicinity of Baghdad, Iraq, and maintained in the laboratory in a similar manner.

The results showed that *S. haematobium* infections distinctly reduced the egg-laying capacity of the snails. There was a 5-fold reduction in the mean number of eggs laid per clutch and more than a 10-fold reduction in the average number of eggs per snail per day in the infected group as compared with the non-infected snails. These differences had a high degree of statistical significance ($p = < 0.001$). (This investigation was made while with the WHO Bilharziasis Control Project in Iraq.)

102. **Life Cycle of *Acanthoparyphium spinulosum* Johnston, 1917 (Echinostomatidae).** W. E. MARTIN AND J. E. ADAMS, University of Southern California.

Johnston described this species from the duodenum of a golden plover, *Charadrius dominious*, collected near Sydney, Australia. A brackish water snail, *Cerithidea californica*, collected at Newport Bay, California, is infected with the parthenitae of *A. spinulosum*. The cercariae, metacercariae, and adults have 23 collar spines arranged in one row. The cercaria has a flame cell pattern represented by the formula $2[(2+2+2+2+2+2+2+2)+(2+2+2+2+2+2+2+2)]=64$. Metacercariae encyst in the muscle and connective tissue of the radula of *C. californica*. Adults were obtained from the small intestine of hatchery-raised chicks.

103. **Studies on the Experimental Infection of Dogs with Three Metacercariae of *Paragonimus westermani* (Kerbert, 1878) Braun, 1899.** M. YOKOGAWA, T. SUGURO, H. YOSHIMURA, AND M. TAUJI, The Chiba University.

In a previous paper, the authors reported on the studies of experimental infections of dogs with a single metacercaria of *P. westermani*. In the present experiments, three metacercariae of *P. westermani* were fed to each of 10 puppies. The puppies were autopsied from 174 to 224 days after feeding.

The results are as follows: (1) All of the puppies were infected, one with a single worm, five with two worms each, and four with three worms each; (2) no cyst was formed in the case of the single worm infection and the trematode recovered was not developed sexually although autopsy was 188 days after feeding; (3) cysts were formed and each contained two, sexually well-developed worms in each case in which only two worms were recovered; (4) cysts were also formed in those hosts from which three worms were recovered. In one instance, three worms were present in one cyst but, while two were well-developed sexually, the third was immature. One cyst also formed in each of the other three infections. Each cyst contained two, sexually well-developed parasites; the third individual was recovered from the pleural cavity of each host and was sexually immature in each instance.

The authors conclude that cross fertilization is necessary for sexual maturation of *P. westermani* and, also, that it is related to cyst formation.

104. **Habitat as a Factor in the Egg Production of the Snail *Bulinus truncatus* from Central Iraq.** H. H. NAJARIAN, The University of Texas, Galveston.

Bulinus truncatus snails, the intermediate hosts of urinary bilharziasis in Iraq, were collected from two localities in central Iraq. One was from an earth canal in Tarmiyah, about 35 miles north of Baghdad, and the other from a shallow ditch in the Alwiyah district of Baghdad. The snails were maintained in a similar manner in the laboratory and egg production was followed on a daily basis without respect to snail size. Of 395 egg clutches examined from the Tarmiyah

snails, the mean number of eggs per clutch was 8.2 ± 3.1. Of 647 clutches laid by the Alwiyah snails, the mean number of eggs per clutch was 12.7 ± 3.5. A statistical analysis of the means showed this difference to be highly significant ($p = < 0.001$). It was concluded that the same species of snail from different habitats may show distinctly different physiological activity. (This study was made while with the WHO Bilharziasis Control Project in Iraq.)

105. **Experimental Studies on the Development of the Resistance of *Oncomelania nosophora* to Sodium Pentachlorophenate.** Y. KOMIYA, K. YASURAOKA, AND Y. HOZAKA, The National Institute of Health, Tokyo.

In order to determine whether acquired resistance would develop and whether a strain of higher resistance exists in *O. nosophora*, experimental studies were carried out.

As to the first item, snails were submerged in 0.0625 ppm. solution of PCP-Na (a sublethal dosage) for 48 hours on every third week and reared in the laboratory. Those in the control group were submerged only in water at the same time. After three immersions the MLD of both experimental and control groups was examined. The results showed that the MLD of the former was 0.463 ppm. and the latter 0.570. The exposure of snails to small amounts of PCP-Na appeared, rather, to make snails more susceptible.

As to the second item, snails were exposed to such selection pressures as LD_{50} and LD_{90} which were determined previously. The MLD of the survivors as well as the controls were as follows: Snails survived in LD_{50}—0.224 ppm.; snails survived in LD_{90}—0.243 ppm.; Control—0.216 ppm. Here no significant difference was observed among the three groups.

106. **The Collagen-Like Nature of Metacercarial Cysts of a New Species of *Ascocotyle*.** H. M. LENHOFF, R. SCHROEDER, AND W. H. LEIGH, University of Miami and Howard Hughes Medical Institute.

A preliminary study of the properties of the metacercarial cysts of a new species of *Ascocotyle* (Leigh, 1959) suggests that the cyst wall may contain a protein related to vertebrate collagens. The cysts were obtained from the conus arteriosus of the Southern Sheepshead Killifish, *Cyprinodon variegatus* (Lacépède). About 500 to 2,000 cysts were found per fish. Nearly the entire population of fish in specific locales was infected. The cysts were isolated by disrupting the conus with a tissue grinder and by discarding fragments of conus as seen in the field of a dissecting microscope. The extraneous protein was removed by washing with 10 percent saline several times. Cyst wall particles, free of the metacercariae, were obtained by disrupting the cysts with a tight-fitting tissue grinder, and by repeated centrifugations and washings. The cysts are slightly oval in shape with a longitudinal axis of 200 microns and a transverse axis of 170 microns. The cyst walls are unusually thick, being 30 to 35 microns. The cyst walls were transparent, pliable, and had a consistency of firm gelatin. On drying the cyst walls became thin and buckled; when placed back into water, the cysts swelled to their original thickness in several seconds. They were resistant to digestion by trypsin, ficin, or papain. A 2-dimensional chromatogram of an acid hydrolysate of the intact cysts showed the presence of a spot having the R_f of hydroxyproline. Chemical analysis demonstrated that an average cyst contained 0.2 microgram of hydroxproline. (The related species *Ascocotyle macintoshi*, which has a similar, but thinner wall, contained 0.1 microgram of hydroxyproline per cyst.) The purified cyst wall particles were also rich in hydroxyproline. This amino acid normally is described as a component of collagens and hitherto has not been reported to occur in the phylum Platyhelminthes. The above results suggest that the cyst wall has a component which belongs to the collagen family of proteins.

107. **On the First Intermediate host of *Paragonimus ohirai* Miyazaki, 1939, and *P. iloktsuenensis* Chen, 1940.** I. MIYAZAKI AND Y. YOSHIDA, Kyushu University and Kyoto Prefectural University of Medicine. (See abstract No. 46.)

108. **Notes on Larval Trematodes From Snails Collected in Gunnison County, Colorado.** G. W. HUNTER, III, AND D. E. BIRKENHOLZ, Rocky Mountain Biological Laboratory, the University of Florida.

Collections of snails were made during 1957, 1958 and 1959 from a series of temporary ponds or "kettles" near Gothic, and a small lake (Grant Lake) near Crested Butte, Colorado. In addition to schistosome dermatitis producing cercariae that were encountered each summer late in July, a number of other groups were found.

In 1959, 773, or 32.7 percent, of 2379 snails of three species were infected as follows: 25 percent of 1618 *Lymnae palustris*, 47 percent of 719 *Physa propinqua* and 60 percent of 43 *Helisoma trivolvis*. These species harbored echinostomes, monostomes, schistosomes, strigeids,

gymnocephalus, and xiphidiocercariae. All three species of snails were infected with strigeid, monostome, and echinostome cercariae. The first two rarely exceeded 10 percent of the snails examined at any one time. On the other hand, the prevalence of cercarial echinostome infections in snails increased from approximately 12 percent of the snails in June to over 50 percent in the first half of August. This suggests that the probable definitive host was a summer resident which arrived early, frequented water and probably raised a brood in or near water. (This investigation was supported by Grant E-1893 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U. S. Public Health Service.)

109. **Studies on Schistosomiasis.** XV. Resistance to *Schistosoma mansoni* in Mice Immunized with *Trichinella spiralis.* C. J. Weinmann, University of Florida.

Since *Schistosoma mansoni* and *Trichinella spiralis* secrete immunologically related substances and serological cross reactions occur (Oliver-González and González, 1949, J. Inf. Dis. **85:** 66–71; Senterfit, 1958, Am. J. Hyg. **68:** 148–155), it was of interest to determine if resistance to infection with *S. mansoni* was influenced by prior immunization with *T. spiralis.*

CFW male mice received a total of five exposures to 100 to 210 larvae of *T. spiralis* per os at monthly intervals. Four weeks after the final immunizing infection, immunized and control mice of the same age and sex were each exposed to 75 ± 2 cercariae of *S. mansoni.* Six weeks later these were sacrificed and their parasite burdens determined. Schistosomes were recovered by perfusion and the number of *T. spiralis* larvae was estimated after digestion of entire eviscerated carcasses.

The 16 test mice harbored a mean *T. spiralis* burden of 28,531 larvae. The mean number of *S. mansoni* recovered from these mice was 17.0 (S.D. = 4.6) and 16.8 percent of the worms were immature. The 14 control mice showed a mean schistosome burden of 13.6 (S.D. = 4.5) with 12.6 percent immature worms. The difference in mean *S. mansoni* burden was not highly significant ($P < 0.06$) but suggests that debilitating effects due to *T. spiralis* infection may have rendered mice more susceptible to subsequent infection with *S. mansoni.* The percentage of immature worms recovered from individual mice in either group was highly variable. (This investigation was supported by Research Grant E-1893 from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service.)

110. **Life Cycle Studies on *Gnathostoma procyonis* Chandler, 1942, a Nematode Parasite of the Raccoon.** L. R. Ash, Tulane University.

The life cycle of *Gnathostoma procyonis* is shown to be similar to that of *G. spinigerum.* Two molts occur in the copepod host and the resulting 3d-stage larva is infective for fish only. Growth and differentiation must occur in the latter before the mammalian host can be infected. Snakes, turtles, and other cold-blooded vertebrates serve as paratenic hosts. Third-stage larvae encapsulate in the muscles of rats and mice without undergoing morphological change. In the definitive host the advanced larval stages undertake a prolonged tissue migration before re-entering the stomach where they reach maturity in approximately 3 months.

G. procyonis differs from *G. spinigerum* in its morphology and behavior in mammalian hosts. In the advanced 3d stage *G. procyonis* is larger, has a lesser number of hooklets on its head-bulb, and does not require migration in the liver of the mammalian host. The posterior half of the adult *G. procyonis* is covered with a series of serrated ridges, and has no naked area on the bursa-like expansion of the male, whereas in *G. spinigerum* the body is devoid of spines over all except the hindmost portion of its posterior half and has a non-spiny Y-shaped area around the anal opening. *G. procyonis* has only the anterior part of its body buried in the stomach nodule, whereas *G. spinigerum* lives entirely within the nodule.

111. **The Effect of Pregnancy on *Aspiculuris tetraptera* Infections in Albino Mice.** M. C. Dunn and H. W. Brown, Columbia University.

Albino mice were used to determine the effect of pregnancy on infcetions with *Aspiculuris tetraptera,* a mouse pinworm. Six-weeks old males and females were randomly grouped so that the latter might be impregnated. Two weeks later, when they were eight weeks old, both the experimental pregnant animals and the non-pregnant controls were given 200 embryonated *Aspiculuris* eggs by stomach tube. After two weeks, the hosts were sacrificed and the parasites counted.

A statistical analysis using Student's "t" test showed there was no significant difference in the number of worms harbored by the control non-pregnant and the experimental pregnant animals. It appears that pregnancy does not have any effect on the worm burden of *Aspiculuris tetraptera* in albino mice.

112. **Factors Influencing the Survival of *Heterakis* and *Histomonas* on Soil.** E. E. LUND, Animal Disease and Parasite Research Division, U.S.D.A.

Poultry yards that had been naturally contaminated with infective stages of *Heterakis* and *Histomonas* were tested semi-annually during 5 years to ascertain the influence of natural factors on infectivity when the soil was not recontaminated. Determinations of infectivity were made by using susceptible poults.

Infections of *Heterakis* acquired by test birds declined 89 percent in the first 5 months (summer) and another 8 percent in the following 7 months. After the second summer, the average infection was 0.8 percent of that at the beginning, 17 months previously. Thereafter, 75 to 96 percent of the birds acquired no worms.

Histomoniasis in test birds declined 35 percent after the first summer, another 10 percent after the first winter, and another 45 percent after the second summer, leaving it 10 percent as prevalent as 17 months earlier. This incidence did not fall appreciably until 4 years had elapsed.

Dehydration, temperate changes causing alternate activation and inactivation, and the action of bacteria, molds, predatory arthropods, and aging all appeared to aid in destroying *Heterakis* eggs at or near the surface of the soil. Dense vegetation tended to retard the influence of some of these factors, and increases in incidence of infection actually occurred in some tests. The study suggested that soil a few inches beneath the surface was the source of the infective stages, which were probably brought to the surface by earthworms, insect larvae, and other agents. These stages were then picked up by test poults. In other test plots, the birds showed increased infections after the soil was cultivated.

113. *Nematospiroides dubius* **in the Mongolian Gerbil.** J. H. CROSS, JR. AND J. A. SCOTT, University of Texas, Galveston.

Infections became established in all of 35 Mongolian gerbils, *Meriones unguiculatus*, following administration by mouth of 100 infective larvae each. The prepatent period in gerbils varied from 10 to 17 days, as compared with 9 to 11 days in control mice. Eggs ceased to appear in the feces of gerbils 21 to 32 days after infection, but they were passed by control mice for as long as 8 months. In a series of 7 gerbils killed from 12 to 16 days after infection the number of worms recovered averaged 52 percent of the larvae given, whereas 86 percent were recovered from 5 parallel control mice. Counts of the eggs passed in the feces for a 24 hour period just before 3 of these gerbils and 2 mice were killed on the 16th day, yielded averages of 710 and 1162 eggs per day, per female worm for the gerbils and the mice respectively.

After the eggs disappeared from the feces of 14 gerbils, an attempt was made to reinfect them, but no eggs appeared in the feces, and no worms were found at autopsy. Some animals were given larvae for a 3d and 4th time, with similar negative results. Infectivity of these various lots of larvae was established by the use of a series of control mice. Evidently, the gerbil is able to acquire a strong resistance to reinfection by this parasite. (This work was supported by Research Grant G–4088 from the National Science Foundation.)

114. **Laboratory Studies of the Rodent Whipworm, *Trichuris muris* (Schrank, 1788).** D. E. WORLEY, J. E. MEISENHELDER, H. G. SHEFFIELD AND P. E. THOMPSON, Research Division, Parke, Davis and Company.

Studies on the biology of the rodent whipworm, *Trichuris muris* (Schrank, 1788) were undertaken to determine its adaptability to various laboratory animals and its suitability for drug evaluation studies on a laboratory scale. Preliminary work on the infectivity of the strain in white mice, DBA–2 hybrid mice, wild house mice, deer mice, white rats, and golden hamsters indicated that only white mice and DBA–2 mice were susceptible. The highest rate of infection was obtained with young DBA–2 mice. Infections in white mice were erratic, with the infectivity rate ranging from 0–40 percent depending on age and source of mice used. Attempts to increase infectivity by various techniques, including the use of decoated eggs for mouse inoculation, were unsuccessful. The influence of concurrent infections with pinworms and tapeworms was examined.

The pre-patent period in mice was approximately 5 weeks. Evidence of host resistance was demonstrated in adult mice by decreases in the rate of infection and percent recovery of *T. muris*. Spontaneous loss of infection occurred after about 60 days in a high percentage of the mice observed. Maximum longevity of infection was approximately three and one-half months. The sex ratio in worms recovered from a few animals was about 1.8 female : 1 male.

The effect of selected anthelmintics, including dithiazanine and pyrvinium, will be discussed.

115. **Changes in Blood of Calves Experimentally Infected with *Ostertagia ostertagi* or *Haemonchus placei*.** F. L. ANDERSEN, D. J. GRAFF, D. M. HAMMOND, P. R. FITZGERALD AND M. L. MINER, Agricultural Experiment Station, Utah State University and the Animal Disease and Parasite Research Division, A.R.S., U.S.D.A.

In 4 experiments with 25 Holstein-Friesian calves, 14 were inoculated at an age of 70 to 110 days with *Ostertagia ostertagi* or *Haemonchus placei* infective larvae and 11 were uninoculated controls; blood samples were collected weekly and the percentages of albumin and the globulins determined with paper electrophoresis; erythrocyte, leukocyte, hematocrit, and hemoglobin determinations were also made. In general, the experiments were continued for 2 to 3 months. In the 7 calves inoculated with about 12,500 to 165,000 *H. placei* larvae (antelope strain) no marked symptoms of parasitism, reduction in weight gain, or changes in serum proteins were observed. However, the hematocrit, hemoglobin and erythrocyte levels decreased. In the 3 calves inoculated with 25,000 to 50,000 *O. ostertagi* larvae, clinical symptoms and a significant reduction in weight gain occurred; the albumin markedly decreased and one or more of the globulins increased. In the 4 calves each inoculated with about 300,000 *O. ostertagi* larvae, the results were similar, except that the controls made abnormally low weight gains and the serum proteins underwent changes like those of the infected animals; the calves were exposed to severe winter weather during much of this experiment. In general, calves infected with either species showed an increase in leukocytes during the first several weeks of infection. No consistent difference between infected and control animals was found in total protein as determined weekly by the biuret method, or in plasma volume as determined biweekly by a modification of Frank and Carr's method. (Supported in part by Western Regional Research Project, W–35, Nematode Parasites of Ruminants.)

116. **Immunization Against the Cattle Lungworm: An Experiment on the Use of Larvae Attenuated by Exposure to X-rays.** J. T. LUCKER AND H. H. VEGORS, Animal Disease and Parasite Research Division, A.R.S., U.S.D.A.

Oral vaccination with 4,000 X-irradiated infective-stage larvae of *Dictyocaulus viviparus* has been reported to almost completely immunize calves against patent infection with this cattle lungworm (Jarrett et al, 1957, Vet. Rec. **69:** 1336–1337; 1958, Proc. Soc. Roy. Med. **51:** 743–744). The X-ray dosage was 40,000 r. The challenge exposure was to 4,000 normal larvae at 8 weeks post-vaccination.

In our experiment, calves were similarly vaccinated with larvae exposed to this same X-ray dosage and, upon more severe and later challenge of any immunity so induced, were found to be as susceptible to *D. viviparus* infection as controls of the same age. The challenge exposure was to 15,000 normal larvae administered 18 weeks after vaccination.

Eight calves were used: when 4.5 months old, each of two (N-larvae group) received 4,000 normal larvae and each of four (X-larvae group) received 4,000 irradiated larvae. The remaining two calves (C-Control group) served as challenge-dose controls. Each of those given normal larvae for immunization had acquired and apparently eliminated a significant patent infection before the challenge dose of larvae was given to all eight calves. A patent infection had not developed in any calf of the X-larvae group. All calves were sacrificed about 5 weeks after challenge. In average susceptibility to lungworm infection on challenge, there was no significant difference between the calves of the X-larvae group and the C-control group as determined by any and all available criteria. The criteria were numbers of first-stage larvae eliminated and clinical behavior post-challenge and gross pathological changes and worm loads found at necropsy. The average numbers of lungworms recovered from the N-larvae group, the X-larvae group and the C-control group were 37, 738, and 800, respectively.

117. **The Effect of Forage Height on the Development of Cattle Nematode Larvae.** H. H. VEGORS, Animal Disease and Parasite Research Division, U.S.D.A.

Two tests were conducted to determine the effect of forage height on the development of infective larvae from roundworm parasite eggs in cattle dung. Each year two plots of short and two plots of tall forage were each equally contaminated with cattle manure. Three weeks later, forage and manure samples were examined for infective larvae. Each plot was then closely grazed by a calf.

The first test was conducted in July and August of 1958, when rainfall was excessive and temperatures were average; equal numbers of larvae were recovered per square foot from tall and short forages. The second test was conducted in May and June 1959, when rainfall was slightly below normal and temperatures were cooler; two-thirds more larvae were recovered per square foot from tall forage plots as from short forage plots.

In the first test, similar numbers of worms were recovered from calves on tall and short forages. However, in the second experiment when the weather was comparatively dry, twice as many worms were recovered from animals grazing tall forage.

Animals on the taller forage yielded more *Haemonchus* spp. and *Trichostrongylus axei* in both tests and more *Ostertagia ostertagi* and *Cooperia* spp. in the second test but not in the first test. More *Nematodirus helvetianus* were acquired by the animals grazing short forage, probably

due to the pick-up of infective eggs. The development of *Oesophagostomum radiatum* was greatly enhanced by forage height as was indicated by the relatively large numbers of this species recovered from calves on tall forage.

118. **The effect of Temperature on Embryonic and Larval Development of Various Species of Cattle Nematodes.** H. CIORDIA AND W. E. BIZZELL, Regional Animal Disease Research Laboratory Substation, A.R.S., U.S.D.A.

Studies on the embryonic and larval development of *Cooperia oncophora, C. punctata, Ostertagia ostertagi, Trichostrongylus axei,* and *T. colubriformis* under constant temperatures of 5, 10, 15, 20, 25, and 32 C, with a relative humidity of about 95 percent, showed that these species developed at the same rate and in similar manner. Daily samples of the egg cultures examined by direct microscopic observations indicated that the rate of development to infective larval state was 7 to 9 days at 25 C and 32 C, 9 days at 20 C, 19 days at 15 C, and about 28 days at 10 C. At 5 C, development ceased after reaching the first larval stage in 24 days. More larvae were recovered from the cultures incubated at 25 C than from those at the other temperatures investigated. Development was accelerated by the introduction of sphagnum moss into the cultures. The acceleration was more evident at lower temperatures, when the developmental period was longer.

Similar work, conducted by placing freshly voided feces from infected calves on outside plots, indicated that the development progressed at a rate influenced by the mean daily temperature obtained from readings taken every two hours. (This work was done in cooperation with the Georgia Agricultural Experiment Station.)

119. **Effectiveness of Three Management Systems on Parasitism in Lambs. I. Clinical Effects of Parasitisms Relative to Exposure and Medication.** K. C. KATES, J. H. TURNER, I. LINDAHL, G. E. WHITMORE, AND F. D. ENZIE, Agriculutral Research Service, U.S.D.A.

Three similar bands of more than 100 lambs each were raised under conditions to induce three levels of parasitism: i.e., low (band 1, kept on a permanent dry lot and fed pellets and hay ad lib), moderate (band 2, rotated on "clean" pastures and fed pellets ad lib), and high (band 3, rotated on contaminated pastures and also fed pellets ad lib). About 20 lambs per band were under parasite surveillance by worm egg counts and hematocrit readings from March to September 1959; similar numbers were necropsied at intervals from June to September. All lambs were on continuous phenothiazine-salt prophylaxis, and those on pasture were given therapeutic treatments with phenothiazine or copper sulfate as was necessary to prevent excessive morbidity and mortality.

Average levels of parasitism acquired by lambs in bands 1, 2, and 3 were insignificant, moderate, and high, respectively, despite the prophylactic and therapeutic medication. The major parasites involved in bands 2 and 3 were *Haemonchus contortus* and *Strongyloides papillosus,* and the clinical picture was mainly that of haemonchosis complicated by strongyloidiasis. Band 1 lambs made ideal growth (terminal weights of remaining lambs averaged 118 lbs. in September); they were unaffected by parasites. Band 2 lambs gained somewhat less than band 1 (terminal weights of remaining lambs averaged 97 lbs in September), and some developed moderate anemia late in the summer; none died of parasitism. Band 3 lambs made the poorest weight gains (terminal weights of remaining lambs averaged 91 lbs in September), and some developed acute anemia; about 9 percent died, primarily from parasitism. Therapeutic treatments were intentionally delayed in bands 2 and 3 to assess the preventive effects of the low-level phenothiazine regimen under the two management conditions. Greater losses presumably would have occurred, particularly from haemonchosis, if medication had not been given.

120. **Effectiveness of Three Management Systems on Parasitism in Lambs. II. Kinds and Levels of Parasitisms Relative to Exposure, Medication, and Weather.** J. H. TURNER, K. C. KATES, I. LINDAHL, G. E. WHITMORE, AND F. D. ENZIE, ARS, U.S.D.A.

Parasitological data consisted of composite and differential worm egg counts made biweekly on 60 lambs, and differential worm counts on 56 necropsied lambs. Clinical coccidiosis did not occur in any lambs, although coccidia were continuously present. Band 1 lambs (on a permanent dry lot) acquired insignificant infections, and need not be considered further.

Band 2 lambs (rotated to "clean" pastures biweekly) principally acquired moderate infections of *Haemonchus contortus* by mid-summer (necropsy worm counts averaged about 600; maximal number in one lamb was 680), and moderate to high infections of *Strongyloides papillosus* in August and September (necropsy worm counts averaged 13,000; maximal number in one lamb was 39,000).

Band 3 lambs (rotated to contaminated pastures biweekly) acquired significant infections

in June of *H. contortus*, which reached a peak in July (necropsy worm counts averaged 13,000; maximal number in one lamb was 28,000). *H. contortus* infection then declined as a result of medication and other factors. *S. papallosus* infection in Band 3 lambs increased to high levels in August and September when high temperature and adequate precipitation prevailed (average necropsy worm counts in mid-August averaged 20,000; maximal number in one lamb was 34,000). Other helminths recovered from Bands 2 and 3 lambs were *Moniezia expansa*, *Nematodirus* spp., *Ostertagia* spp., *Trichostrongylus* spp., *Cooperia curticei*, and *Oesophagostomum venulosum*. Not a single specimen of *O. columbianum* was recovered.

Weather conditions during the experiment were favorable for the development, survival, and transmission of *H. contortus* and *S. papillosus*. Medication partially controlled infection with *H. contortus*, but apparently it had no effect on *S. papillosus*.

A phenothiazine-salt regimen alone did not prevent acute haemonchosis from developing in some lambs that were grazed on contaminated pastures.

121. **Effectiveness of Three Management Systems on Parasitism in Lambs. III. Resistance of *Haemonchus contortus* to Phenothiazine.** F. D. Enzie, M. L. Colglazier, G. E. Whitmore, and D. E. Thompson, U.S.D.A.

Four days before the lambs (except those in band 1) and ewes were turned on their respective pastures, 25 g of phenothiazine (N. F. grade, average particle size about 10 microns) were given to ewes weighing 100 pounds or less and 37.5 g to all others. Substantially smaller doses have been reported by several investigators to be adequate for the highly effective removal of *Haemonchus contortus* from sheep. Lambs and ewes in all bands were given continuous access to 1:9 phenothiazine-mineral mixtures.

Band 1 lambs (dry lot) acquired insignificant infections and required no therapeutic medication. Band 2 lambs (rotated on "clean" pastures) and band 3 lambs (rotated on contaminated pastures) acquired, respectively, moderate and high levels of parasitism, the infections consisting predominantly of *H. contortus*. In late summer and early fall, both bands also developed heavy infections of *Strongyloides papillosus*. Therapeutic medication against *Haemonchus* was intentionally delayed in bands 2 and 3 to assess as definitively as possible the efficiency of the preventive measures used in the two management systems. Development of clinical parasitism, however, first in band 3 and somewhat later in band 2, necessitated therapeutic medication. Band 3 lambs were given three 25-g doses of phenothiazine at 4-week intervals, beginning in early July; on the third treatment occasion, similar doses were given to band 2 lambs. Satisfactory response was not obtained in either group. In early September, band 3 lambs were removed to dry lot; lambs in both bands 2 and 3 were then started on a regimen of periodic treatment with copper sulfate solution. After three doses, at intervals of 10 days to 3 weeks, the lambs in both bands were given a final drench of 25 grams of phenothiazine. There was no appreciable response to any of these treatments.

Available but incompletely analyzed data from critical trials with experimental infections of this strain of *H. contortus* indicate a resistance to the action of phenothiazine comparable to, if not greater than, that reported by Drudge and his co-workers (1957 a,b,c; 1959) in Kentucky. Additional trials will calibrate the degree of resistance, investigate the phenomenon in other *Haemonchus* strains, and assess the anthelmintic action of a purified grade of phenothiazine against resistant strains.

122. **The Effect of Early Weaning on Acquisition of Gastrointestinal Nematodes by Lambs.** N. D. Levine, W. F. Schaeffler and J. Szanto, University of Illinois.

Lambs ordinarily become parasitized with gastrointestinal nematodes by ingesting larvae while grazing on pastures which have been contaminated by their mothers. During the 1958 grazing season, an attempt was made to determine the effect of early weaning and separation of lambs from their ewes on the acquisition of gastrointestinal nematodes. One group of 10 lambs was weaned abruptly on April 28 and placed on an 0.5 acre brome grass-alfalfa pasture, while 10 other lambs were placed on a 1.25 acre pasture along with their 10 mothers. Both groups were kept on pasture until October 1. Fecal counts were made weekly of strongyloid nematode (mostly *Haemonchus contortus*) eggs, *Strongyloides papillosus* eggs, and coccidian oocysts, and the lambs were weighed monthly.

There were no significant differences between the strongyloid and *S. papillosus* egg counts, coccidian oocyst counts or weight gains in the 2 groups of lambs. The mean strongyloid egg count never rose above 1750 eggs per gram (e.p.g.) in either group, which was very low as compared with previous years. This was probably due to the fact that more pasturage was provided than the animals could handle, so that they grazed it very lightly.

The experiment was repeated during the 1959 grazing season. One group of 10 lambs

was weaned abruptly on May 1 and placed on an 0.62 acre brome grass-alfalfa pasture, while 10 other lambs (mostly twins of the weaned ones) and their 10 ewes were placed on a 1.0 acre pasture. By July 6 the pastures had been grazed off almost completely and had to be increased to 1.25 and 1.5 acres, respectively. The experiment was continued until September 28. Fecal egg and oocyst counts were made and the lambs weighed as before.

The lambs grazing with their mothers became very heavily parasitized, and 8 of them died between July 1 and August 11. The mean strongyloid egg count reached a peak of 73,900 e.p.g. in July. The weaned lambs never became heavily parasitized with nematodes, their mean strongyloid egg counts never exceeding 3500 e.p.g. during the whole season. They did not gain weight well, however, due to the poorness of the pasture after the spring and early summer.

123. **Rate of Body Weight Gain of Rats Infected with** *Trypanosoma lewisi.* D. R. LINCICOME, R. N. ROSSAN, AND W. C. JONES, Howard University and Naval Medical Research Institute.

Molecular exchanges between foreign autonomous cells living in the cellular and tissue environment of their hosts have not been studied extensively. Identification of these exchanges might furnish evidence of the chemical adjustments necessary to permit the intimate parasitic relationship.

The rat and its usual blood parasite,*Trypanosoma lewisi* were employed to test an hypothesis that during infection of the rat the parasite contributed to the host's metabolic processes. Rate of body weight gains of weanling and young mature female albino rats was the criterion.

Six experiments were completed using 66 control and 130 experimental animals. Of two experimental groups in each protocol, one received a smaller number of trypanosomes than the other. Body weights were usually recorded every third day.

Data were expressed as increases over the original body weights. They were plotted and lines fitted by the method of least squares. The curves drawn in this manner indicated a linear pattern of growth of both infected and non-infected rats through the second and in some instances the third week. The slopes of the curves indicated that inoculated animals had a faster rate of gain of body weight than uninfected ones. In some experiments, animals receiving a larger number of trypanosomes in the inoculum appeared to gain weight faster than those receiving a smaller number.

Beyond the third week the curves indicated a decline in growth rate. Fitting of the data by inspection gave additional evidence of a higher rate of growth of infected animals.

The presence of autonomous foreign cells in the blood stream of rats, under the experimental conditions employed, is therefore apparently associated with accelerated growth of the host. Whether this is due to a metabolic contribution of the parasite to the host remains to be demonstrated.

124. **Induction of Development of** *Trypanosoma lewisi* **in the Mouse by Heterologous Sera.** D. R. LINCICOME AND E. H. FRANCIS, Howard University, Washington, D. C., and the Medical Research Institute, Bethesda, Md.

The rat trypanosome, *Trypanosoma lewisi,* will develop in the heterologous mouse host only when small quantities of normal homologous rat serum are inoculated daily into the mouse (Lincicome, 1955, 1957, 1958). The trypanosome populations in the mouse blood stream develop and terminate in a manner similar to those in the rat, and the parasite may be serially maintained by syringe passage to subsequent mice provided a small supplement of rat serum is given daily to each mouse.

The present study reports the presence in heterologous sera of growth-promoting factors for this trypanosome that are either identical with or similar to those in rat serum.

Sera from swine, chickens, cattle, dogs, man, hamsters, guinea pigs, and rabbits have been tested in mice for their abliity to support development of the trypanosome taken directly from the homologous host. The swine, chicken, cattle, man and dog sera are ineffecive. Sera of the hamster, guinea pig and rabbit supported development of the parasite, but to a lesser degree than rat serum.

The minimal daily supplement of rat serum necessary to sustain a growing population of *T. lewisi* in the blood stream of the mouse was determined as a standard for quantitative study of the titer of growth-promoting substances in other sera. A volume of rat serum containing 2.5 mg of serum protein daily appeared to be the smallest supplement. With reference to this minimum, rabbit serum was 40 percent and guinea pig and hamster sera were respectively 50 percent and 80 percent as effective as homologous control serum in supporting the development of this parasite in an heterologous environment.

125. **Attempts at Interspecies Genetic in Trypanosomes.** Y. U. AMREIN AND C. D. WIENS, Pomona College, Claremont, California.

Methods are given for the testing of genetic transfer between *Trypanosoma cruzi* and *T. lewisi* in the rodent and triatomid hosts. Preliminary results seem to suggest that, under the conditions of these experiment,s no interspecies transfer takes place. (This investigation was supported in part by Research Grant E-2915 from the Public Health Service.)

126. **A Medium for the Axenic Cultivation of the Oral Flagellate** *Trichomonas tenax*. L. S. DIAMOND, Laboratory of Parasitic Diseases, National Institutes of Health, Public Health Service.

An anaerobic fluid medium has been devised for the axenic cultivation of *Trichomonas tenax*. To date two strains (Hs-2: NIH, Hs-4: NIH) have been successfully cultivated. Multiplication of the flagellates through 10 transplants served as the criterion of success (Cailleau, 1937, Ann. Inst. Past. **59:** 137). One strain is now in the 20th transfer, the other in the 14th.

Trichomonads grown in association with *Trypanosoma cruzi* in the medium of Phillips (1951, Am. J. Trop. Med. **31:** 290) were used to initiate the cultures. Those trypanosomes introduced along with the oral flagellates died out rapidly, usually in the 2d or 3d subculture.

The medium consists of an autoclaved portion composed of trypticase, tryptose, yeast extract, glucose, L-cysteine hydrochloride, ascorbic acid, and 0.05 percent agar in buffered saline, and a non-autoclaved portion composed of horse serum and chick embryo extract (CEE).

CEE is processed at 0 C and is the centrifugate which results from spinning at 850 *g* for 20 minutes a blendorized preparation of 12-day-old embryos in buffered saline (1 g: 2 ml). It is stored at −20 C until used. CEE is employed in concentrations of 5 to 10 percent (v/v) and is essential for axenic growth. It fails to support growth even in concentrations up to 30 percent if after thawing from the frozen state it is subjected to further centrifugation of 2000 *g* for 30 minutes.

127. **Cultivation of** *Histomonas melagridis* **in Modified Tissue Culture Media.** E. LESSER, Animal Disease and Parasite Research Division, A.R.S., U.S.D.A., Beltsville, Maryland.

Histomonas meleagridis has been successfully cultured in a variety of tissue culture media ("199," "NCTC 107," "NCTC 109," and Eagle's), each modified by the addition of (1) cream sterilized by passage through a Selas filter, (2) an antibiotic-treated nutrient broth culture of turkey cecal bacteria, and (3) large quantities of penicillin G potassium, dihydrostreptomycin sulfate, and mycostatin. The cultures were sealed with vaseline. The modified "199" medium was used for all routine cultivation.

The histomonads have now been maintained through more than 90 consecutive transfers, and some of them have been subcultured more than 55 consecutive times without evidence of the presence of viable bacteria.

When Seitz filtrates of the bacterial cultures, autoclaved bacterial cultures, or bacterial cultures heated at 56 C for 60 minutes were used in lieu of the antibiotic-treated bacterial cultures, no histomonad growth occurred. It appears, therefore, that one or more heat labile intracellular factors present in turkey cecal bacteria are essential for growth of *H. meleagridis* in any of the above media.

128. **The Cultivation of** *Blastocrithidia leptocoridis* **(McCullough).** F. G. WALLACE AND M. I. DYER, University of Minnesota, Minneapolis.

The species of *Blastocrithidia* Laird (1959, Can. J. Zool. **37:** 749–52) from Hemiptera have proved far more difficult to isolate in culture than most other insect trypanosomatids. After many unsuccessful attempts over the past year we have obtained a vigorous culture of *B. leptocoridis* from the box elder bug, *Leptocoris trivittatus*. The medium used was Difco brain heart infusion agar to which 10 percent of fresh rabbit blood was added. Each 5 ml slant of this blood agar was covered with 0.5 ml of brain heart infusion broth with penicillin and streptomycin added. The insects were washed in zephiran chloride and dissected with aseptic precautions. When bits of infected gut were inoculated into the above described tubes there was only weak growth, which failed in subcultures. Successful cultivation was achieved only when capillary tube cultures were used for preliminary isolation. The capillary tubes contain $.08 \pm .01$ ml of the same medium. Inoculation of test tube cultures from rich capillary tube cultures results in exponential multiplication.

The hypothesis that the ratio of the number of organisms to the amount of medium is a critical factor suggested itself. Inoculation of 100 organisms or less into test tube cultures (5.5 ml of medium) produced only weak multiplication while inoculation of 1000 or more produced

vigorous cultures. Growth curves of this species resulting from varying inocula are compared with grow h curves of other more easily cultivated insect flagellates. (This investigation was supported by grant E–2373 C1 from the National Institute of Allergy and Infectious Diseases, Public Health Service).

129. **Reticulo-Endothelial Blockade in Experimental Chagas' Disease.** F. C. GOBLE AND J. L. BOYD, CIBA Pharmaceutical Products Inc.

Although the importance of the reticulo-endothelial system in *Trypanosoma cruzi* infections has been recognized for many years, and some experimental procedures, such as cortisone administration and X-irradiation, have been shown to be effective in reducing resistance to the parasite, splenectomy has been consistently reported to have no influence on the infection in laboratory animals, and most experiments in the use of reticulo-endothelial blockade have been inconclusive. Denison (1943, Am. J. Hyg. **38:** 178) reported that blockade with trypan blue reduced the lytic titers of sera of rats infected with an avirulent strain of *T. cruzi*, although the effects on susceptibility, as evidenced by parasitemia, were not striking. Friebel (1952, Arch. Exp. Path. Pharm. **216:** 536), however, found that the administration of trypan blue to mice with *T. cruzi* caused a drop in parasitemia on the 11th day of infection.

Using colloidal thorium dioxide, which has, in other host-parasite systems, consistently been found to depress the RES by blockade, it has been demonstrated that infections with the "Corpus Christi" strain of *T. cruzi*, resulting from inoculation of culture forms of low virulence, can be enhanced. In a group of DBA mice, blockaded before infection, only one-ninth remained negative throughout the test. Deaths occurred on the 11, 15, 17, 30, and 33d days bringing the number of survivors at 60 days to four-ninths as compared with eight-ninths in the control group. Only one of the controls became positive. Similar experiments in CF1 mice also resulted in enhanced infections.

130. **Studies on the Immunology of Experimental Chagas' Disease.** L. NORMAN AND I. G. KAGAN, Communicable Disease Center, Atlanta, Ga.

Eighteen experiments were performed with seven animal strains of *Trypanosoma cruzi* (isolated from opossum, raccoon and skunk captured in Georgia and Florida), three human strains of *T. cruzi*, *T. duttoni* and *T. lewisi* to test the protective action of an initial infection with these organisms when challenged 4 to 6 weeks later with a virulent lethal strain of *T. cruzi*-Tulahuen (Chilean origin). All the animal strains of *T. cruzi* which are avirulent in mice and other animals, protected mice against the lethal challenge. Two experiments with non-*cruzi* trypanosomes (*T. duttoni* and *T. lewisi*) failed to protect mice.

Mice infected with *T. cruzi*-FH5 (opossum strain) were challenged from 2 to 365 days (14 experiments) after initial infection to test the duration of immunity. Mice infected for 2 to 3 days were not protected; 4 to 21 days partially protected and for 28 to 365 days completely protected against the lethal action of *T. cruzi*-Tulahuen. The Chilean strain of *T. cruzi* kills control mice in approximately 12 days. Infection with the initial avirulent strain was confirmed by culture in all animals prior to challenge. Serum from mice infected with FH5 strains or from mice surviving challenge, protected normal animals from a lethal challenge provided the challenge was administered shortly after the transfer of serum. Experiments to determine whether virulence of the lethal strain is permanently reduced by passage through immune mice are in progress.

131. **Agar Diffusion Studies of Species Specificity of *Balantidium coli*, *B. caviae* and *B. wenrichi*: Preliminary Studies of Antigenic Properties.** S. KRASCHENINNIKOW, University of Pennsylvania, AND E. JESKA, Pennsylvania Department of Health Division Laboratories.

Balantidium species from pig, guinea pig, and spider monkey (*Ateles* sp.) were isolated from intestinal contents, purified, then stored in distilled water at −20 C. Lyophilization was carried out at reduced pressure at −72 C.

Ten-mg fractions of each species' material were homogenized in 1 ml of 0.85 percent saline emulsified in equal parts with Freund's complete adjuvant, and were injected subcutaneously into rabbits. After the immunization program the rabbits were bled to obtain antisera. Assays of antigenic properties were performed by Ouchterlony's method.

The antigens of *B. coli*, *B. caviae* and *B. wenrichi* were tested against antisera, normal sera and saline controls, in agar plates. The bands appeared only between the wells which contained the antigens and homologous and heterologous antisera. *B. coli* antigen reacted against its antiserum with four bands, the two nearest the serum well having a very heavy precipitate. This antigen reacts with *B. caviae* antiserum by providing four precipitation bands with the heavily precipitated ones nearest the serum well. *B. wenrichi* antiserum yields only one band with this

antigen. The *B. caviae* antigen produces precipitation bands with its homologous serum. The center band shows a very heavy precipitation. Only two very light bands with *B. coli* antiserum are formed but there are indications of the inhibition of band formation in the vicinity of this well. This antigen also produces one precipitation band with *B. wenrichi* antiserum. Two bands appeared with both *B. coli* and *B. caviae* antisera in the zone of the *B. wenrichi* antigen. This antigen gives only one band with its antiserum. (Research supported by U. S. Public Health Service Grant E-999.)

132. **The Free Amnio Acids in** *Trichomonas foetus.* A. E. JOHNSON, Animal Disease and Parasite Research Division, A.R.S., U.S.D.A., Logan, Utah.

Free amino acids contained in water extracts of the protoplasm of *Trichomonas foetus* were identified by means of one- and two-dimensional paper chromatography. Bacteria-free cultures of these parasites, originally isolated 6 months previously from an Aberdeen-Angus bull, were used.

The trichomonads, cultured in agar-free CPLM medium containing 15 percent bovine serum, were harvested by centrifugation, washed in at least four changes of sterile physiologic salt solution, and then lysed in distilled water, using one to two million organisms per ml, to release the free amino acids. Cell fragments were removed by centrifugation, proteins by the use of ion exchange resin, and carbohydrates by 80 percent ethanol. Before testing, the extracts from about 2,500 million trichomonads were dried by evaporation, then redissolved in 0.5 ml of 10 percent isopropyl alcohol.

Unidimensional ascending chromatograms were made on Whatman No. 1 filter paper in butanol-acetic acid-water (12:3:5). For identification, amino acids were stained by the polychromatic stain of Moffat and Lytle.

Nyhydrin-positive areas obtained on two-dimensional ascending chromatograms, using butanol-acetic acid-water and phenol-ammonia as solvents, were identified by comparing their Rf values with those of known amino acids.

The amino acids identified by the unidimensional chromatograms were alanine, glycine, valine, the leucine-isoleucine complex, cystine, lysine, aspartic acid, glutamic acid, proline, tyrosine, and phenylalamine. Serine, arginine, threonine, and cysteine were tentatively identified by the two-dimensional chromatograms.

133. **Evaluation of Techniques for the Demonstration of** *Trichomonas vaginalis.* B. S. HAYES AND E. KOTCHER, The University of Louisville.

Identification of *T. vaginalis* by Papanicolaou staining is not satisfactory because of the absence of morphological details. Wet preparations and culture techniques may be used, but are time consuming and may not reveal the trichomonads. This study is an attempt to develop a more accurate and less time consuming procedure for the detection of *T. vaginalis* and to compare its efficiency with the above diagnostic procedures. Specimens of vaginal secretions from 225 women were examined for *T. vaginalis* by wet preparation, Papanicolaou staining, culture in sodium thioglycollate, and fluorescent antibody techniques. *T. vaginalis* antiserum was prepared in the rabbit and the antiserum was conjugated with fluorescein isothiocyanate. The direct technique was used. Of the 225 specimens examined, 33.3 percent were positive for *T. vaginalis* by the wet preparation technique, 40 percent by the culture technique, 13 percent by the Papanicolaou staining technique and 39.6 percent by the fluorescent antibody technique. The results indicate that the culture and fluorescent antibody procedures were superior to wet preparations and Papanicolaou stains. The fluorescent antibody procedure required approximately 2 hours for demonstration of *T. vaginalis,* while 48 to 72 hours were required for demonstration by culture methods.

134. **Quantitative Aspects of** *Eimeria acervulina* **Infections.** S. M. KRASSNER, The Johns Hopkins University, School of Hygiene and Public Health.

Chickens, 1 week to 2 months of age, were infected with one million sporulated oocysts of *Eimeria acervulina.* Until 72 hours postinfection the parasite could be found only in the anterior part of the small intestine, but afterwards it was present all along the intestine. The number of merozoites of the first and second generations per infected cell averaged 13 and 19 respectively. This is lower than estimates made by previous authors.

Infected cells do not hypertrophy as stated by other workers. They are simply expanded by the projection of the parasite against the cell wall. Cells were studied using the fluorescent stain, acridine orange. The cytoplasm of the parasite fluoresced red-orange while its early nucleus fluoresced light orange. When the merozoite is mature, the nucleus fluoresces yellow.

Observations on gut washings showed the oocysts to be free in periodic peaks during the

infections. The highest peak appeared at about 102 hours postinfection. The peaks then afterwards decreased in size.

Chicks became better hosts of the parasite as they grew older up to the age of 2 months. This was tested by the oocyst yield of infected birds. Observations on oocyst content of the entire gut showed that birds which had been previously infected became resistant to further infections. This differs from earlier work in which difficulty was experienced in obtaining chicks resistant to *Eimera acervulina*. (This investigation was carried out during the tenure of a predoctoral fellowship from the National Cancer Institute, United States Public Health Service.)

135. **Oocyst Production in Subclinical Infections with Coccidia in Cattle.** W. C. MARQUARDT, Montana Veterinary Research Laboratory.

As a part of a study on the etiology of winter coccidiosis in cattle, oocyst production was studied in normal, subclinical infections. In the first study, a total of 181 fecal oocyst counts were made on five weaned beef calves starting late in October and ending during the first week of March. Three species, *Eimeria ellipsoidalis*, *E. bovis* and *E. canadensis*, comprised 72 to 86 percent of all oocysts. The agent causing winter coccidiosis, *E. zürnii*, comprised 6 to 16 percent of all oocysts. *E. zürnii* occurred in 17 to 45 percent of all samples, *E. ellipsoidalis* in 44 to 75 percent, *E. bovis* in 83 to 97 percent and *E. canadensis* in 44 to 58 percent of all samples.

In the second study, four dairy calves were raised in a barn under conditions of minimum exposure to coccidia. During January 1959, when these calves were approximately 3 months of age, two of them were removed and placed in a corral with two 8-month-old Hereford calves. Fecal oocyst counts were made three times a week on all calves until March 30. Oocyst numbers in the isolated dairy calves and in those exposed to the older Hereford calves remained uniformly low. Despite higher total counts and the appearance of *E. zürnii* in relatively large numbers on two occasions in the Hereford calves, there was no indication of transmission of *E. zürnii*, or any other species, to the younger calves.

136. **Comparison of the Effects of Three Isomeres of Hydroxybenzoic Acid on *Trypanosoma lewisi* Infection.** E. R. BECKER, Arizona State University, Tempe.

It has been reported that the oral administration of salicylates in sufficient dosage interferes with the immune response of the rat that inhibits the reproduction of *Trypanosoma lewisi* (Becker and Gallagher, 1947, Iowa St. Coll. J. Sci. **21**: 351–362). The same effect can be produced by the intraperitoneal injection of 45 mg of sodium salicylate in aqueous solution daily commencing the beginning of the 3d day and continuing through the 7th or 8th day. It can also be produced by the oral administration of 45 to 60 mg daily of sodium salicylate dissolved in aqueous 3 percent sodium bicarbonate solution; also, of the same weight of ortho-hydroxybenzoic acid (salicylic acid) dissolved in the same medium whose alkalinity increases the solubility of this chemical. Meta-hydroxybenzoic acid or para-hydroxybenzoic acid, when solubilized with 3 percent sodium bicarbonate or when administered in partial suspension after warming, however, do not produce the salicylate effect on ablastin (the anti-reproduction antibody of Taliaferro). (This investigation was supported in part by research grant E-2975 from the National Institutes of Health, Public Health Service.)

137. **Some Factors Involved in Excystation of the Sporozoites of Three Species of Sheep Coccidia.** J. C. LOTZE AND R. G. LEEK, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

Studies on the excystation of sporozoites from oocysts of ovine coccidia under natural conditions indicate that three important factors are involved, namely, (1) the sporozoites must be in proper physical condition to exert physical force against the protective coverings of the sporocyst and the oocyst and perforate them, (2) the parasites must be quickly aroused to activity when the oocyst arrives in the small intestine, and (3) before entering the small intestine the oocyst wall must be conditioned so that sporozoite-activator(s) in the small intestine can rapidly penetrate the oocyst and reach the sporozoites. Evidence supporting these conclusions was found in (a) the in vitro excystment of sporozoites of three species of ovine coccidia studied, *Eimeria faurei*, *Eimeria arloingi*, and *E. ninae-kohl-yakimovi*, when their oocysts, after sporulation in tap water at room temperature (62 F to 82 F), were incubated in plain sheep, pig, chicken, or rabbit bile at 103.5 F for 5.5 to 6 hours and sporozoites began to excyst from the sporocysts, and (b) the finding that time required to produce the same results under similar conditions was reduced to 0.5 to 2.5 hours by a 12- to 17-hour "pretreatment" consisting of first incubating the oocysts, at the same temperature, in either Seitz-filtered human saliva or Seitz-filtered 1.0 percent

solution of powdered commercially prepared steapsin made up freshly in distilled water each time before use.

138. **Experimental Investigations of the Influence of Humidity on the Optimum Temperature Range of Medically Important Bluebottles and Mosquitoes (Calliphoridae and Culicidae).** W. G. SHULZE, Zoological Institute of Münster University, Westphalia, Germany. (Abstract not received.)

139. **An Effective Method for the In Vitro Culture of** *Hymenolepis diminuta.* A. K. BERNTZEN, Portland State College.

Larvae of *Hymenolepis diminuta* were isolated from infected bettles and grown in vitro to adults which contained developing onchospheres. After dissection, the excised larvae were washed several times in sterile Tyrodés solution, and cultured at 37 C by aseptic technique in an apparatus designed for continuous flow of medium over the worms. Several types of media were used. The growing worms were measured on the 3, 4, 7, 10, 12, and 15 days of culture. The length measurements on the 15th day varied from 15 to 25 cm. The experiment was terminated on the 15th day, and the worms were fixed in hot acetic acid and formalin mixture, and then stained accordng to the method of Tahmisian for structure study. Data on the types of media, rates of growth, and design of the culture apparatus are presented.

This method of in vitro culture may serve as a useful tool for parasitologists, since it assures complete control of environment and media, as well as observation of the growing worms. It is also useful in that the media, which have been modified by the worms metabolic processes, may be continuously collected for analysis. This work was supported in part by a grant from Portland State College. (This abstract, received late, was added to the page proof.—Editor.)

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C. W. STILES*	1925	H. J. VANCLEAVE*	1938–41		1956–59
	1929–32	E. R. BECKER	1939–43	E. E. BYRD	1952–55
E. E. TYZZER	1925–26	E. W. PRICE	1939–46	C. A. HERRICK*	1952–55
M. C. HALL*	1926–29		1947–50	A. MCINTOSH	1953–56
W. G. SMILLIE	1926–27	C. G. HUFF	1940–46	R. V. MOORE	1953
H. B. WARD	1926–29		1948–51	A. O. FOSTER	1954–55
F. D. BARKER*	1927–30	H. W. STUNKARD	1940–43		1957
J. H. ST. JOHN*	1927–28	D. L. AUGUSTINE	1941–44	L. E. ROZEBOOM	1954
W. H. TALIAFERRO	1928–31	W. H. WRIGHT	1942–48	L. O. NOLF	1955–58
A. C. CHANDLER*	1929–30	R. M. CABLE	1942–45	L. A. STAUBER	1955–58
	1936–39		1954–57	F. J. KRUIDENIER	1956–59
W. B. HERMS*	1930–33	G. F. OTTO	1942–48	W. TRAGER	1956–57
B. SCHWARTZ	1930–33	H. W. MANTER	1944–46	G. H. BALL	1958–
L. R. CLEVELAND	1931	H. W. BROWN	1944–47	D. A. PORTER	1958–
W. W. CORT	1931–38	G. R. COATNEY	1945–48	P. P. WEINSTEIN	1958–
H. E. EWING*	1931–32	T. W. M. CAMERON	1946–48	R. P. BAUGHAM	1959–
E. C. FAUST	1931–34	C. B. PHILIP	1947	L. J. OLIVIER	1959–
	1938–41	M. D. YOUNG	1947–50	R. M. LEWERT	1960–
J. F. KESSEL	1932–35	P. D. HARWOOD	1948–51	C. P. READ	1960–

* Deceased